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F-94-R

# ILLINOIS RIVER FINGERNAIL CLAM TOXICITY STUDY

July 1, 1990 - December 31, 1991

Final Report to

Illinois Department of Conservation

by

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## ACKNOWLEDGEMENT OF SUPPORT

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## EXECUTIVE SUMMARY

A filtering performance bioassay was developed for the fingernail clam, *Musculium transversum*, a dominant bottom-dwelling organism in many waters of the midwestern United States, and a key link in food chains leading from organic matter in water and sediment to fish and ducks valued by humans. The bioassay was used with a battery of standard bioassays to assess the toxicity of porewaters obtained from sediments of the Illinois River and its associated canals (known collectively as the Illinois Waterway), where fingernail clams and other benthic macroinvertebrates died out in 1955-1958 and have not recolonized, despite the availability of seed populations in tributaries and isolated refugia within the river. Inhibition of filtering performance was easily measured with relatively simple equipment available in most laboratories and proved to be directly related to the concentration of a reference toxicant, sodium cyanide. The filtering response of *M. transversum* was consistent with the mortality response of a standard reference zooplankter, *Ceriodaphnia dubia*: both organisms exhibited no response to porewaters obtained from sediments from the lower Illinois River or from a reference site on the Upper Mississippi River, whereas porewaters from 7 of 13 upstream sites were toxic to *C. dubia* and 12 of the sites inhibited filtering performance of the clam. The responses of the clam and zooplankter were inconsistent with the responses of standard reference organisms, a freshwater alga (*Selenastrum capricornutum*) and a marine bacterium (*Photobacterium phosphoreum*), which were actually stimulated by some porewaters that were toxic to the clam and zooplankter. In view of the great physiological differences among plants, bacteria, and animals, this result was not too surprising; e.g., ammonia is toxic to aquatic animals at concentrations that can be used as a nitrogen source by plants and some bacteria.

The toxic porewaters were treated to remove certain classes of toxicants, then retested for toxicity with *C. dubia*. Based on these tests and chemical analyses of the porewaters, the toxicity in the upper Illinois Waterway is attributable largely to ammonia, with some marked local toxicity attributable to petroleum-based hydrocarbons.

During the course of this study *M. transversum* recolonized some areas in the upper Illinois Waterway where it had been absent and declined at the reference site and several other places on the Upper Mississippi River. The baseline filtering rates of clams from different sources varied, probably depending on their previous exposure to stress, including ammonia, in the waterways. Clams from some sources on the lower Illinois River and the Chicago waterways either did not respond or were actually stimulated by added ammonia concentrations of up 0.09 mg/l (un-ionized $NH_3$-N), indicating that they either were too stressed to respond or had been exposed to ammonia long enough to have been selected for ammonia tolerance. In contrast, added ammonia significantly inhibited the filtering of clams from the reference site in the Upper Mississippi River and two sites in the Chicago Sanitary and Ship Canal. In the short span of two months, we observed abundant clam populations disappear at some sites in the Chicago waterways, leaving only dead shells. Although a general recovery in fingernail clam populations in the Illinois Waterway does seem to be underway, this recovery apparently is set back by episodes of sediment toxicity. An investigation similar to the one reported here should be undertaken in the Upper Mississippi River where clam populations at several sites have undergone sharp fluctuations recently.

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## ACKNOWLEDGEMENT

Many people contributed to this project. Dr. Anthony A. Paparo, Department of Zoology and School of Medicine, Southern Illinois University at Carbondale, did much of the early development of a variety of methods for assessing responses of clams and mussels to contaminants. Dr. Philippe Ross, who at the time was in the Center for Aquatic Ecology at the Natural History Survey in Champaign-Urbana, assisted Dr. Paparo in preparing the initial proposal. Ms. Diane Dillon, Mr. Jeffrey Arnold, and Mr. Kurt Pemberton provided valuable technical assistance at Western Illinois University, as did Ms. Louann Burnett at the Natural History Survey in Champaign, and Mr. K. Douglas Blodgett at the Natural History Survey's Long Term Resource Monitoring Station in Havana. Mr. Arnold assumed much responsibility for running bioassays and analyzing results toward the end of the project period. Mr. Blodgett wrote the LOTUS program for converting total ammonia to un-ionized ammonia and prepared the maps of the study area and several of the figures used in this report. Ms. Camilla Smith provided secretarial assistance at the River Research Laboratory of the Stephen A. Forbes Biological Station in Havana. The research could not have been done without the laboratory facilities, office space, and equipment provided by Dr. Richard V. Anderson and the Department of Biological Sciences at Western Illinois University--to both we express our great appreciation. We also thank the following people at the Illinois Department of Conservation: the project officer, Mr. William Bertrand, Administrator of the Streams and Rivers Program; Mr. Larry Dunham, Administrator Research and Operations; and Mr. Michael Sweet, Federal Aid Coordinator in the Division of Resource Management Finance. The Acting Head of the Center for Aquatic Ecology of the Illinois Natural History Survey, Dr. Daniel Soluk, also reviewed the report. Finally, this research would not have been done without the grant from the Federal Aid in Sport Fish Restoration Program, administered by the Region 3 U.S. Fish and Wildlife Service office in Twin Cities, MN.

# 1.0 INTRODUCTION

## 1.1 Importance of Fingernail Clams and this Research

Fingernail clams (family Sphaeriidae) are dominant bottom-dwelling animals in many waters of the midwestern United States. They are found in major rivers (Gale 1969), lakes (Emmling 1974), and bottomlands (Hubert 1972). They are key links in food chains leading from nutrients in water and mud to fish and ducks which are utilized by humans, including the highly-valued channel catfish, *Ictalurus punctatus*. Fingernail clams filter algae, bacteria and organic matter from water. Because the clams are small (<15mm or 0.6 in. long when full-grown) in comparison to mussels (family Unionidae), they are readily consumed by benthivorous fish. One species of fingernail clam, *Musculium transversum*, is especially important as a food item for fish (Ranthum 1969 and Jude 1968 and 1973) because it occurs at densities up to 100,000/$m^2$ or 83,600/$yd^2$ (Gale 1969), has a fragile, easily-masticated shell, and contains 13% protein and 2% fat (dry weight basis, Thompson and Sparks 1978). Also, *Musculium transversum* has been used as an indicator species for the benthic food base, representing other small mollusks, including snails, as well as mayflies and other burrowing aquatic insects that were virtually eliminated from certain reaches of the Illinois River by 1958 (Sparks 1984; Paloumpis and Starrett 1960).

*Musculium transversum* has not recolonized the Illinois River in its former numbers since 1958, despite the fact that seed populations are available in tributaries and the clam is capable of quickly repopulating an area because it has a very short life cycle--33 days in midsummer (Gale 1969). Our working hypothesis was that if we can find out what prevents the fingernail clam from recolonizing portions of the Illinois River where it was formerly abundant, we will have a strong indication of what killed the other species. Once the inimical factors are identified, they could perhaps be eliminated or controlled, so that the benthos of the river would recover, to the benefit of benthivorous fishes and diving ducks.

Declines of fingernail clams have not been limited to the Illinois River. Wilson et al. (unpublished manuscript) concluded that populations of fingernail clams declined significantly in five of eight navigation pools on the Upper Mississippi River for which historical data existed, and warned that these decreases could signal a large-scale deterioration in the health of this ecosystem. Results of research on clam declines in the Illinois River might help explain or even forestall similar declines in other rivers, such as the Upper Mississippi River.

## 1.2 Relationship to Other Research

Previous research demonstrated that the inimical factor was associated with sediment, rather than with the water itself, because fingernail clams survived in Illinois River water that was filtered to remove sediment (Sparks, Sandusky and Paparo 1981), and bulk sediments obtained from backwaters and floodplain lakes along the river were toxic, as measured by a clam gill bioassay (Sparks, Sandusky and Paparo 1983; Blodgett et al. 1984).

In 1988, we obtained funding from the Illinois Department of Energy and Natural Resources and the Illinois Environmental Protection Trust Fund to use a battery of standard bioassays to assess the pattern of toxicity in the river sediments and, if possible, to identify the toxic agents by treating the sediments to remove certain contaminants and then reassaying them to check for reductions in toxicity. The USEPA (Mount and Anderson-Carnahan 1988 and 1989; Mount 1988) refers to these procedures as Toxicity Identification Evaluation (TIE). The pattern of toxicity might indicate potential sources: e.g., if toxicity increased upstream, the Chicago urban area would be suspect. If toxicity increased below major tributaries that drain agricultural land in the central part of the state, then agricultural sources would be suspect. The clam gill bioassay is not a standard bioassay and so was not included. The purpose of F-94 was to use the fingernail clam bioassay in conjunction with the standard bioassays, to insure that the results were relevant to the problem of the fingernail clam decline and subsequent failure to recolonize. It is also useful to know whether the standard reference organisms are in fact good surrogates for key organisms that are important in particular aquatic ecosystems.

## 1.3 History of the F-94 Project

The clam gill assay was developed by Dr. Anthony Paparo, Department of Zoology and School of Medicine, Southern Illinois University at Carbondale. He became incapacitated during the course of the project and could no longer continue the bioassays. His bioassay procedure requires special equipment and skills because it involves microsurgery on the small clams and microscopic observation of particle transport rates and rates of beating of cilia on the exposed gills of the clams. The project was amended to develop a much simpler functional assay to substitute for the more complex technique, and the location of the work was shifted from Southern Illinois University to Western Illinois University in Macomb. The simpler procedure measures the ability of intact clams to filter yeast suspensions (which serve as food for the clams) from water, following exposure to test solutions. Both methods are described in this report because some interesting results were obtained with the original method.

Two other surprises occurred during the project, one pleasant and one unpleasant, that required modifications to our original plans. The unpleasant surprise was a collapse in fingernail clam populations and persistent low numbers during the period 1988-1992 in Pool 19 of the Upper Mississippi River, which had always been the source of our bioassay clams and of our supposedly uncontaminated control sediment. For a time we could not obtain enough clams to run bioassays, until we located another source, but with much lower densities, in Swan Lake on the lower Illinois River. The pleasant surprise was the reappearance of fingernail clams in several locations in the Illinois Waterway including portions of the canal system in and near Chicago. Since clams reappeared in some locations where our initial results had indicated toxicity, we ran a series of bioassays with stocks of clams from different parts of the river to determine whether some stocks were more tolerant of toxic sediments than others, perhaps having undergone

selection for resistance through a long history of exposure to local toxicants.

## 1.4 Objectives

In summary, the objectives of the project, as modified by the circumstances above, were: (1) to develop a simple functional bioassay for the fingernail clam, *Musculium transversum*, (2) to compare the new bioassay to the previous technique (the gill assay) and to bioassays with standard reference organisms, (3) to use the bioassay, in conjunction with the standard bioassays, to assess the pattern of toxicity in sediments of the Illinois River, (4) to use the bioassay, again in conjunction with standard bioassays, to identify toxic agents in the sediments, and (5) to determine whether stocks of clams from different parts of the river were differentially sensitive to the toxic agents. Objectives (1), (2), (3), and (5) were met. Objective (4) was partially met, by testing treated (5 treatments to reduce toxicity) sediment porewater from one location on fingernail clams, as well as on a standard reference organism, the water flea, *Ceriodaphnia dubia*, whose responses more closely paralleled those of the clam than any of the other three reference species we tested. The water flea was used for all the other toxicity identification procedures because the new clam assay was not developed as quickly as we had hoped and because of additional delays in finding a new source of clams following the decline in our original source population in the Upper Mississippi River.

# 2.0 METHODS

## 2.1 Site Description

Today's Illinois Waterway is approximately 327 miles (526 km) long connecting Lake Michigan and the Chicago-Joliet metropolitan area with the Mississippi River and the agricultural heartland, near Grafton, Illinois (Figure 2.1). The headwaters are in the highly industrialized Chicago area where the flow of the Chicago River was reversed to carry wastes away from Lake Michigan into the Illinois River via the Chicago Sanitary and Ship Canal and the downstream portion of the Des Plaines River (Figure 2.2). The Calumet Sag Channel enters the Sanitary and Ship Canal near Lemont. The Illinois River proper begins with the confluence of the Des Plaines and Kankakee rivers, and flows through a predominantly agricultural drainage, although the industrial city of Peoria is situated approximately mid-way along the waterway.

Locations on the waterways are designated by river mile as recorded in river charts prepared by the U.S. Army Corps of Engineers (1987) and by markers along the waterways, starting with mile 0.0 at the confluence with the Mississippi and proceeding upstream to Chicago. The following abbreviations are used in the text, figures, and tables to identify reaches of the waterway, and stations are identified by reach abbreviation and river mile:

| | |
|---|---|
| IR | Illinois River proper |
| DP | Des Plaines River |
| CS | Calumet Sag Channel |
| SS | Chicago Sanitary and Ship Canal |
| CR | Chicago River |

The one reference station on the Upper Mississippi River is located 377.0 miles above the confluence with the Ohio River and is designated MR 377.0. The locations of the sample stations are given in Table 2.1 and Figures 2.1 and 2.2. In accordance with Corps of Engineers terminology, the designation "left bank" or "right bank" assumes the observer is facing downstream.

## 2.2 Sampling Design

Nineteen sampling stations were established throughout the Illinois Waterway (Figures 2.1 and 2.2). Samples were collected from 15 stations from November 1989 to June 1990, and from all 19 stations from November 1990 to June 1991 (Table 2.1).

## 2.3 Sample Collection Procedures

2.3.1 Sediment Collection. It is important to limit the disruption of the sediment so that toxicity evaluations are conducted under conditions that closely match the *in situ* conditions (ASTM 1991). The most appropriate sediment sampling device is study specific. Sediment corers generally disrupt the sediment little but collect a limited sample volume (ASTM 1991). This study employed a battery of bioassays as well as the TIE procedures, all of which used sediment

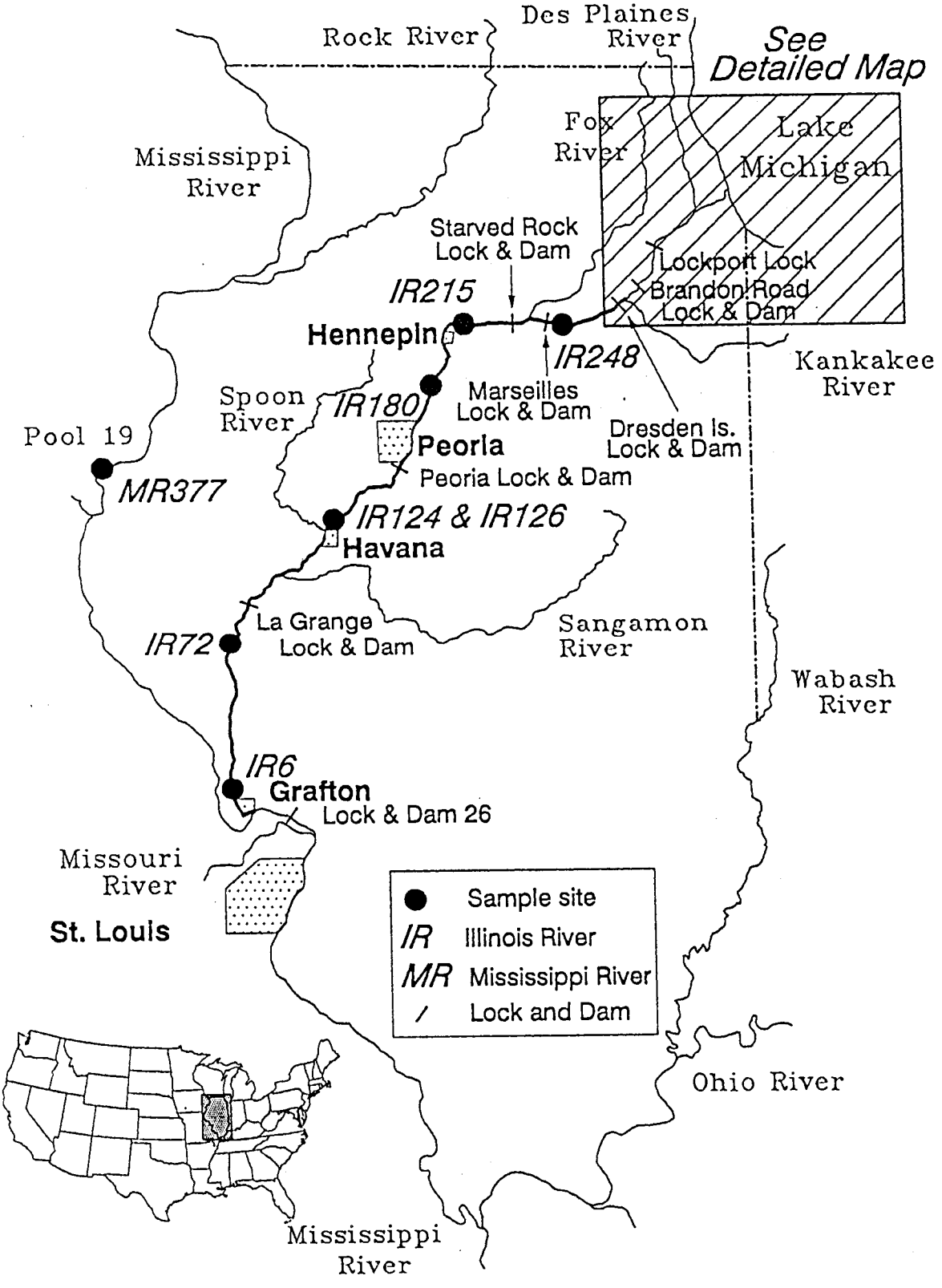


Figure 2.1. Location of sediment sampling stations on the Illinois Waterway. Stations are identified according to river miles: Illinois River miles (IR) start at Grafton at mile 0.0 and proceed upstream to Chicago. A reference station was established on the Mississippi river (MR), 377 miles above the confluence with the Ohio River.

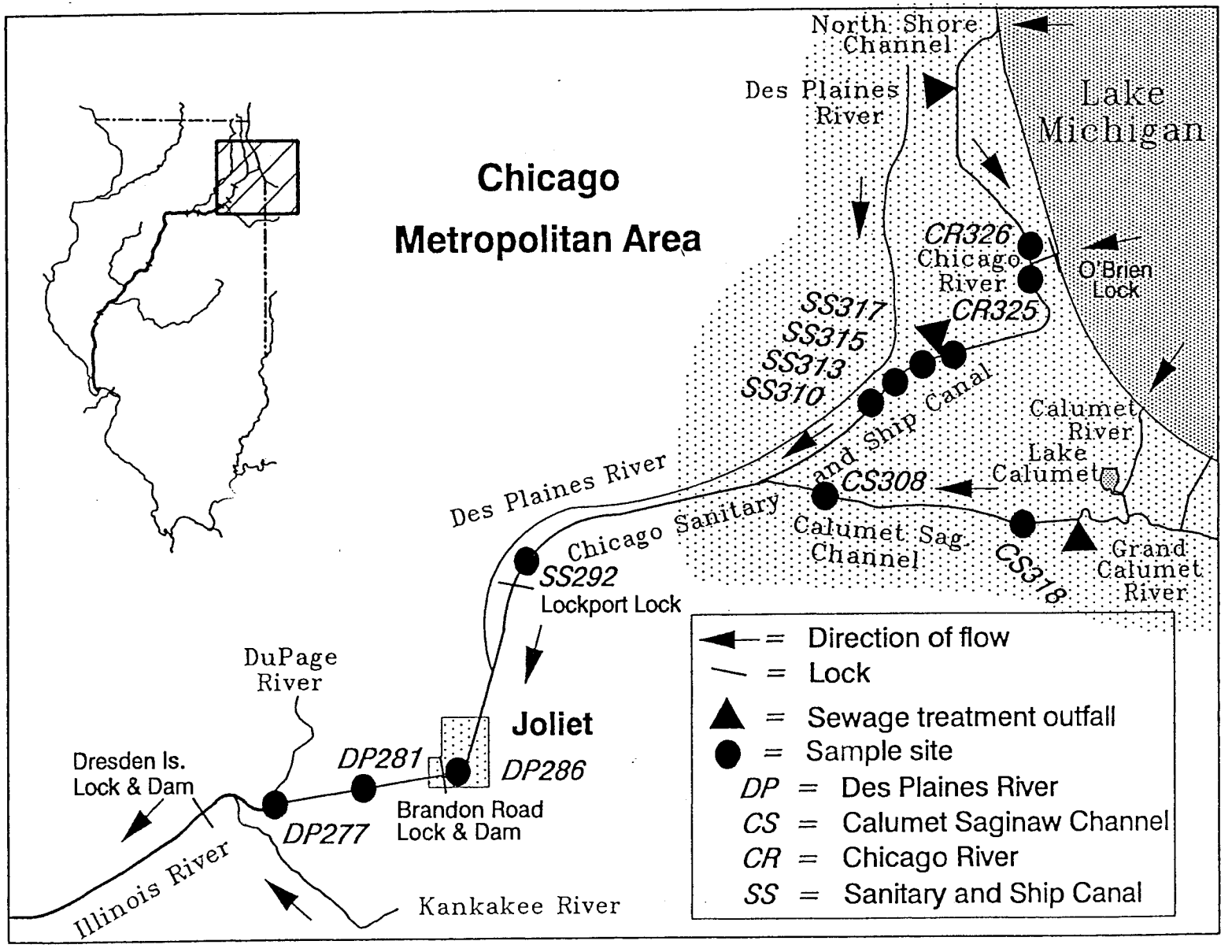


Figure 2.2. Location of sediment sampling stations in the Chicago-Joliet area. Stations (black circles) are identified according to distance (in miles) upstream from the confluence with the Mississippi River at Grafton, and according to the name of the reach. DP = Des Plaines River. SS = Chicago Sanitary and Ship Canal. CS = Calumet Sag Channel. CR = Chicago River. The location of the major sewage treatment plant outfalls in the Chicago area are noted: NSTP = northern sewage treatment plant, SWTP = southern sewage treatment plant, and CTP = Calumet treatment plant. Arrowheads indicate usual direction of flow in the waterways.

Table 2.1. Location of sampling stations.

| River Mile | Description |
|---|---|
| CR 326.4 | North Branch of Chicago River at Michigan Avenue Bridge |
| CR 324.8 | South Branch of the Chicago River at Harrison Street Bridge |
| CS 318.5 | Upstream of Division Street Bridge on Calumet Sag Channel |
| CS 307.4 | Upstream of 104th Street Bridge on Calumet Sag Channel |
| SS 317.0 | ~5 m (16 ft) from left bank |
| SS 315.3 | ~25 m (82 ft) from left bank |
| SS 313.0 | ~2 m (6.5 ft) from right bank downstream of Route 171 Bridge |
| SS 310.0 | ~10 m (33 ft) from left bank upstream from Justice Navigation Light |
| SS 292.2 | 10 m (33 ft) upstream of sunken barge and 30 m from right bank |
| DP 286.3 | Left bank ~300 m (984 ft) upstream of Brandon Road Lock and Dam |
| DP 281.1 | ~30 m (98 ft) from left bank across from Olin Chemical |
| DP 277.0 | Upstream of Du Page River Daymark ~500 m (1,639 ft) from right bank |
| IR 248.2 | ~100 m (328 ft) upstream of Ballards Island |
| IR 215.0 | Center of Turner Lake |
| IR 180.0 | Upper Peoria Lake, south of Chillicothe |
| IR 125.5 | SE Corner of Lake Chautauqua |
| IR 72.0 | Center of Meredosia Lake |
| IR 6.0 | Entrance to Swan Lake |
| MR 377.0 | Montrose Flats, Pool 19, Mississippi River |

Note: The Illinois Waterway includes the Illinois River (IR), Des Plaines River (DP), Chicago Sanitary and Ship Canal (SS), Chicago river (CR), and Calumet Sag Channel (CS). The mileages start at IR 0.0 at the confluence with the Mississippi and proceed upstream to Chicago. Mileages on the Upper Mississippi River (MR) start at the confluence with the Ohio. "Right" and "left" assume the observer is facing downstream. m = meters.

porewater. The volume of porewater needed for this work made the use of sediment corers impractical. We used a 25.4-cm (10-inch) Ekman dredge that works well in the soft to semi-soft sediments that characterize the Illinois Waterway and collects a relatively large sample volume (ASTM 1991).

The sampler was rinsed with river water at the site prior to sediment collection. The sample was placed in prewashed (Biosoap wash, ultrapure water rinse) high density polyethylene containers. High density polyethylene containers are relatively inert and are optimal for samples contaminated with a variety of chemicals (ASTM 1991). The containers were filled completely to achieve zero sample head space. Sample containers were placed on ice as soon as possible following collection (never exceeding 2 hours). Samples were transported to the laboratory and stored at 4°C (39.2°F) for no more than two weeks.

2.3.2 Extraction of Sediment Porewater. We used sediment porewater in our toxicity tests. Numerous studies (Adams, Kimerle and Mosher 1985; Swartz et al. 1985; Knezovich and Harrison 1988; Connell, Bowman and Hawker 1988; Swartz et al. 1988, Di Toro et al. 1992) have shown that porewater is an appropriate surrogate for bulk sediment. Porewater can be collected from sediment samples by several methods: centrifugation, squeezing, suction, and equilibrium dialysis (ASTM 1991). Centrifugation is generally used if large volumes of porewater are required (Edmunds and Bath 1976). Constituents such as salinity, dissolved inorganic carbon, ammonia, sulfide, and sulfate are generally not affected as long as oxidation is prevented; however, dissolved organic carbon (DOC) and dimethylsufide may be significantly reduced using this method (Howes, Dacey and Teal 1985). Sediment porewater was extracted by centrifugation at 4000 g (g = the acceleration due to gravity) at 4°C (39.2°F) for 45 minutes. The supernatant porewater was siphoned through a Nitex 110-mesh screen and stored with zero head space at 4°C (39.2°F) in a decontaminated cubitainer for a maximum of 1 week. The time from collection to testing ranged from 1 to 6 days, and averaged 2.6 days for all sediments.

2.3.3 Collection of Surface Water. Surface water samples were collected just prior to collection of sediment. Surface water was collected from approximately mid-depth in the water column using a Van Dorn sampler. Samples were placed in pre-cleaned cubitainers and immediately placed on ice. Surface water samples were stored at 4°C (39.2°F) for a maximum of one week.

## 2.4 Chemical Analyses

Routine chemical measurements were taken on both surface water and porewater samples. Samples were brought to ambient temperature (20-24°C, 68-75°F) prior to making the following measurements in the laboratory:

| SURFACE WATER | PORE WATER |
|---|---|
| Dissolved Oxygen | Dissolved Oxygen |
| pH | pH |
| Conductivity | Conductivity |
| Alkalinity | Alkalinity |
| Hardness | Hardness |
| Total Ammonia-N (ammonia measured as nitrogen, N) | Total Ammonia-N |
| | Total Cl (chlorine) |
| | $H_2S$ (hydrogen sulfide) |
| | Sulfide |

Dissolved oxygen was measured using a standard Y.S.I. Model 57 oxygen meter with a Y.S.I. Model 5739 probe. Temperature and pH were measured using a Jenco Microcomputer pH-Vision 6071 pH meter with a temperature-compensating Ross electrode filled with Ross reference electrode filling solution #81-00-07. The pH meter was calibrated with Cole-Parmer pH 4.01 and 7.00 standard buffer solutions before use. Specific conductance was measured using a Y.S.I. Model 35 Conductance Meter with a Y.S.I. Model 3401 probe. Total alkalinity was measured using the ASTM (1982) standard titration method. Fifty ml (1.5 fl oz) of sample water was stirred by a magnetic stirrer at medium speed while the sample was titrated with 0.02 N $H_2SO_4$ to a pH of 3.7. Milliliters of titrant were multiplied by 20 to calculate mg/l total alkalinity as $CaCO_3$. Total hardness, as $CaCO_3$, was measured by the Hach burette method (Hach 1985) adapted from the EDTA titrametric method of APHA (1976). Total ammonia nitrogen was determined using the Hach Nesslerization method (adapted from APHA 1976). The method was modified by adding 1 drop of Rochelle salt solution prior to the Nessler reagent to prevent precipitation of magnesium hydroxide in the sample cell in water samples where hardness exceeded 100 mg/l $CaCO_3$. Results are reported as mg/l total ammonia nitrogen. Total residual chlorine was determined by the DPD colorimetric method, sulfide by the methylene blue method, and hydrogen sulfide by the lead sufide method, following the Hach Water Analysis Handbook (1985), which is adapted from APHA (1976). All instrumentation was calibrated prior to testing.

We intended to calculate the fraction of the total ammonia that existed in the un-ionized state during the toxicity tests (see below) using aqueous ammonia equilibrium calculations and knowing the pH and temperature (Emerson et al. 1975). In aqueous ammonia solutions an equilibrium exists between ammonia in the highly toxic un-ionized form ($NH_3$) and ammonia in the relatively nontoxic ionized form ($NH_4^+$). The dominant factor regulating the equilibrium between the two forms is pH, with the temperature having a lesser effect. We were not able to calculate un-ionized ammonia concentrations in the toxicity tests because the pH of the porewater drifted slightly during the tests. Temperature was held constant. However, our subsequent analysis of the correlation between toxicity and total ammonia is justified because the initial pHs of the samples were similar (6.5-7.25) and all drifted in a similar manner, so the average un-ionized ammonia concentrations during the tests were some consistent fraction of the total ammonia concentrations.

Measurements of total organic carbon (TOC) were performed on bulk sediment samples. The results are expressed in percent organic carbon.

## 2.5 Collection and Maintenance of Fingernail Clams

Barely enough fingernail clams were obtained from Pool 19, Mississippi River (MR 377.0) at the beginning of the project to run the gill assay described below. Another source of clams eventually was located in Swan Lake on the lower Illinois River, near IR 6.0, and these were used throughout most of the project period to develop the bioassay that measured filtering performance and to test the toxicity of porewater from sediments. Finally, near the end of the project period, we collected clams from MR 365.5, IR 5.1, and two sites in the Chicago waterways (Sanitary and Ship Canal, SS 292.7; Calumet Sag Channel, CS 318.5; see Figure 2.1 and 2.2), to compare the sensitivity of clams from the different sites to ammonia. We also recorded observations on the number of live clams and dead shells at several other sites where we did not obtain clams in sufficient numbers to run bioassays.

Sediments were collected with an Ekman dredge and sieved through a wash bucket with a 500-micron screen. Clams were hand-picked from the screen and kept in a cooler with aerated river water until delivered to the laboratory. They were held in aquaria in river water and fed a suspension of the green alga, *Selenastrum capricornutum*, and a yeast-Cerophyl-trout food (YCT) that is normally used for *Ceriodaphnia dubia* (NETAC 1989b). Clams were used within 14 days of capture. They were placed in synthetic dilution water 1 hour prior to being tested. The total hardness of the dilution water was adjusted to match that of the sediment pore water, by adding the appropriate salts to increase hardness, or by dilution with distilled, deionized water to reduce hardness.

Although every effort was made to obtain clams in the field that were approximately the same size, and again to choose clams of the same size from the holding aquaria for the experiments, we were forced to use a wider range of sizes than we wished, because it took so much effort to find clams at the field sites. The shell lengths of clams used in these experiments ranged from 5.9 to 12.0 mm (0.236-0.480 in), averaging 9.0 mm (0.360 in).

## 2.6 Fingernail Clam Gill Bioassay.

Dr. Anthony Paparo conducted the gill assays on clams kept for at least seven days in circulating, aerated river water in an Instant Aquarium (temp. 17-20$^{0}$ C 62.6-68$^{0}$ F, pH 7.5) in a laboratory at Southern Illinois University in early April 1989. Before each experiment, clams 5.0-10.0 mm in length were placed in finger bowls of the same river water. The posterior adductor muscles were cut, and each gill with its branchial nerve, visceral ganglion, and a piece of adductor for support, were isolated. The ganglion/nerve/gill preparation was pinned to a rubber mat glued to the bottom of a Petri dish containing river water, and the dish was placed in a holder fastened to the adjustable stage of a microscope. Under magnification (100X), the gill was seen to consist of numerous parallel gill filaments. Three major types of ciliated cells were clearly distinguished: frontal, laterofrontal and lateral.

The rate of beating of the lateral cilia was measured. These cilia beat in such a way that metachronal waves appear to travel in opposite directions along the two sides of each gill filament.

The optical field was selected for observation by measuring 2.0 mm in an anterior direction from the visceral ganglion. A field of view contained about 50 gill filaments which were grouped for ease of observation into three vertical columns. By moving the microscope stage each gill filament was followed from its dorsal attachment at the axis to its free ventral end. Each column was subdivided into four horizontal rows, demarcated by their fixed number of interfilamentary junctions, from dorsal to ventral end. The rate of ciliary beating in beats per second was measured by synchronizing the rate of flashing of a calibrated stroboscopic light (used in place of the substage lamp) with the rate of beating of the cilia. Synchronization was achieved when the metachronal wave appeared to stand still. The rate was then read from a digital flash rate on the strobe. Measurements were made from dorsal to ventral border, and from left to right across the field: 12 sets of measurements for each gill preparation, which then were averaged.

Sediment suspensions were prepared by adding the same arbitrary volume of spoon-mixed wet sediment from each site (collected from Pool 19, Upper Mississippi River, on 21-22 March 1989, and from the Upper Illinois River 28-29 March) to one liter of standard molluscan physiological solution. The test solutions then were diluted with the physiological saline solution until they all had similar particle concentrations, as determined by counting the particles with a hemocytometer. Control suspensions were prepared to match the average particle concentration of the test solutions, only using yeast instead of sediment. Blodgett (1983) reported the following ranges in mean sediment and yeast suspensions from similar experiments:

| | Particle Size $\mu$ m ($.04 \times 10^{-3}$ in) | Density mg/l (ppm) | Concentration $10^6$ particles/l ($1.06 \times 10^6$ particles/qt) |
|---|---|---|---|
| sediment | 2.9 - 7.2 | 29.3 - 78.4 | 2.1 - 3.9 |
| yeast | 6.8 - 7.2 | 46.3 - 59.1 | 2.5 - 2.7 |

The suspensions were pumped across the Petri dishes containing the gill preparations via a four-channel, variable-speed pump with a flow rate of about 0.5 ml/min (.015 fl oz/min). The planetary gear mechanism of this pump ensured minimum pulsing and stable drift-free flow, permitting accurate measurement of ciliary movement. A positive displacement piston metering pump with micrometric adjustment removed the solution from the other side of the dish, thereby maintaining a continuous flow of solution across the dish. The temperature was maintained at $20^{\circ}$ C ($68^{\circ}$) by circulating water from a constant temperature bath at 2.0 ml/min (.06 fl oz/min) through stainless steel tubing in the movable microscope platform that held the Petri dish. Dissolved oxygen remained at 8.0 ppm during the exposure periods, and the pH was monitored and adjusted to 7.5 by adding acid or base, if necessary.

Ten gill preparations were used for each sample tested: five controls (exposed to yeast suspensions) and five experimental

preparations (exposed to the sediment suspensions). Measurements were made prior to introduction of the suspensions and again after 30 minutes of exposure to the suspensions. The measurements were averaged across the five animals in each group, and the results expressed as a percentage increase (stimulation) or decrease (inhibition) in ciliary beating rates of the experimental animals relative to the controls. In 20 previous control tests with equivalent yeast suspensions the gill preparations showed a net change of 0 ciliary beats/sec $\pm$ a standard deviation of 1.25 during the 30-min. exposure period (Blodgett et al. 1984). Therefore, a reduction in the ciliary beating rate of the test gills was regarded as a response to toxicity in the sediments and not to the particle concentrations.

## 2.7 Fingernail Clam Filtering Bioassay.

2.7.1 Rationale and General Procedure. The fingernail clam filtering assay developed in this study is based on observations by Aldridge, Payne and Miller (1987), Sparks and Sandusky (1983), Sparks, Sandusky and Paparo (1981), and Anderson, Sparks and Paparo (1978) that stresses, including toxicants, impair the ability of bivalves to filter particles from water (including food particles, such as yeast, on which the clams feed). Only the 1990-1991 porewater samples were evaluated using this assay because it was not fully developed until late 1990. A detailed description of the general procedure follows.

Fingernail clams are first exposed to the porewater sample for one hour. They are then removed from the test solution and given a filtering performance test, which consists of placing them in a yeast suspension in dilution water (10% by weight, based on dry weight of yeast) and allowing them to filter for one hour (Figure 2.3). Two controls are used: the first consists of the yeast suspension alone and is used to determine the change in concentration due to settling of the yeast. The second control determines the baseline filtering rate of clams exposed for 1 hour in clean, uncontaminated water.
The yeast concentrations are measured indirectly at the beginning and end of the filtering period, by measuring light transmission in water samples from the test chambers with a spectrophotometer, then using a regression equation that relates light transmission to measured yeast concentrations:

Y = yeast concentration (mg/l)

T = % transmittance of light

$$Y = 676.378 - 6.788\ T$$

The filtering rates of the exposure and control tests are determined by taking the initial yeast concentration minus the final concentration minus the amount settled divided by the weight of the test organisms.

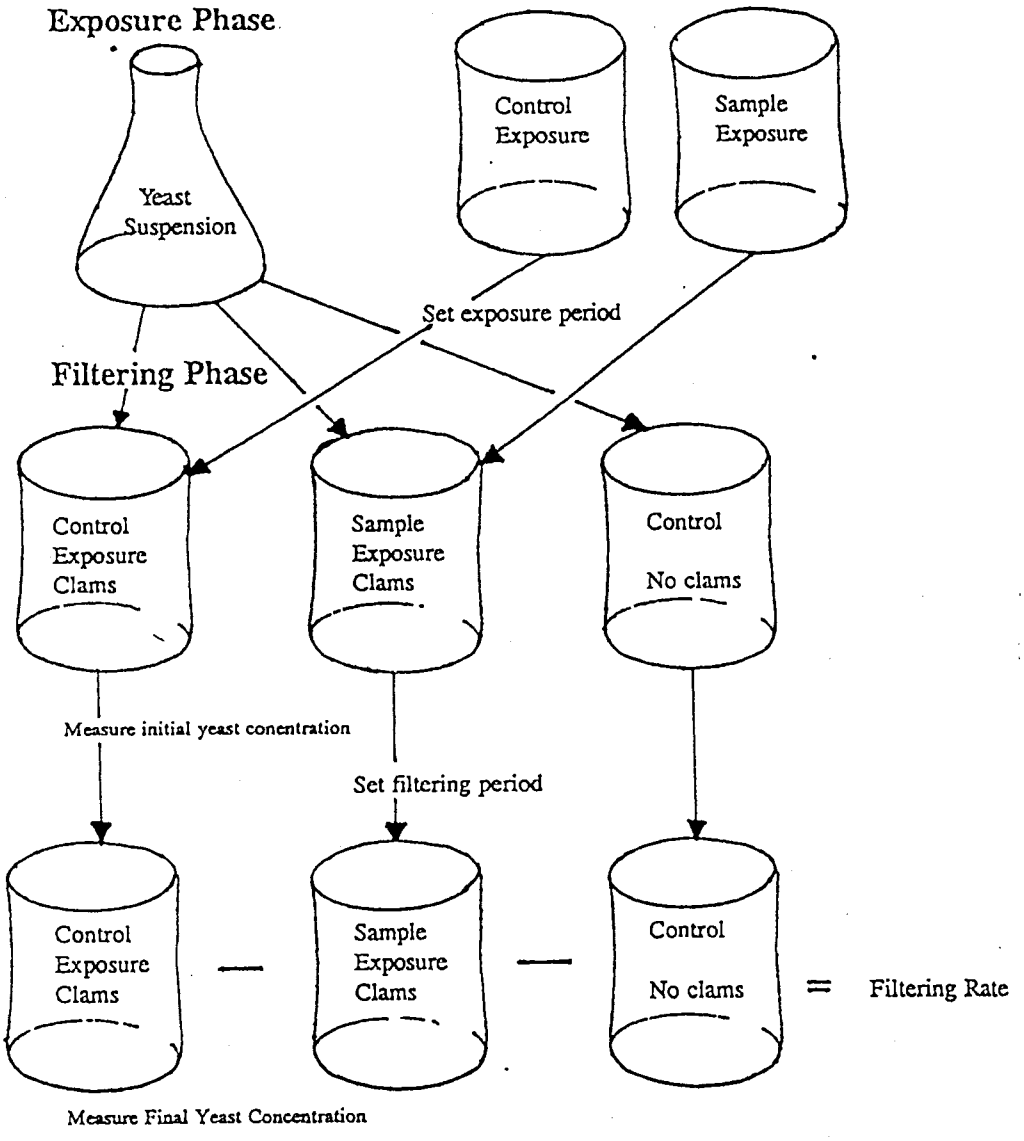


Figure 2.3. Steps in fingernail clam filtering bioassay.

Filtering rates are expressed as the concentration of yeast filtered per unit weight of organisms per unit time.

$C_i$ = initial concentration of yeast

$C_f$ = final concentration of yeast

$W$ = live weight of clams, in g (grams), x 0.035=oz

$C_s$ = change in yeast concentration due to settling

$$\frac{C_i - C_f - C_s}{W} = \text{filtering rate in mg (milligrams) yeast/g clam/hour}$$

(mg x $3.527 \times 10^{-5}$ = oz)

The organism weights are for whole live animals, with shells, blotted dry with Chem Wipes, a tissue type of absorbent material. Five clams were used in each test chamber.

2.7.2 Assessment of Response. The filtering rate of clams exposed to the test solutions is then compared to the control to determine the degree of response. Since there is natural variation in the filtering rate of healthy clams, we decided to calculate a threshold value for a change in filtering rate that we would consider to be beyond the range of normal variation. We took the maximum range of variation in filtering rate observed in all the control trials and added two times the standard deviation. This threshold amounted to 10.6% of the mean control value, so filtering rates would have to decline more than 10.6% in relation to the control before the test solution would be regarded as toxic (inhibitory). Likewise, the filtering rate would have to increase more than 10.6% before the test solution would be classified as stimulatory.

## 2.8 Reference Toxicant.

It is useful to have a bioassay with a graded response so that sampling sites can be ranked according to relative toxicity, thus revealing spatial and temporal trends that might indicate sources. "All or none" (death or survival, toxic or nontoxic) responses are less useful for this purpose. In order to determine whether filtering inhibition was proportional to toxicant concentration, we tested a reference toxicant, cyanide, over a range of concentrations (1-100 mg/l added as sodium cyanide salt) known to bracket a lethal level for fish and other standard test species.

## 2.9 Sensitivity of Clams from Different Sites to Ammonia.

This component was added late in the project when it became obvious that fingernail clams were reappearing in portions of the Illinois Waterway where our Toxicity Identification Evaluation (TIE) procedures had identified toxicity attributable to ammonia. Fingernail clams from the various sources were exposed to dilution water as a control and to three concentrations of ammonia in dilution water, to

determine whether clams from different sites differed in their tolerance for ammonia. The ammonia was added as ammonium chloride, measured in the test solutions as described in section 2.3, and the un-ionized ammonia nitrogen, $NH_3$-N, concentrations calculated and reported.

2.10 Reference Bioassays.

2.10.1 Reference Species and Responses. We compared the response of fingernail clams, *Musculium transversum*, to the responses of four standard reference species, as determined in the DENR project (Sparks, Ross and Dillon 1992). The standard bioassays employed the following organisms: the marine bacterium, *Photobacterium phosphoreum* (Microtox™), the freshwater alga, *Selenastrum capricornutum*, the rotifer, *Branchionus calyciflorus*, the daphnid, *Ceriodaphnia dubia*, and the sphaerid clam, *Musculium transversum*. The Microtox™ assay measures the luminescence of *P. phosphoreum* (Bulich, Greene and Isenberg 1981). Inhibition of this luminescence is considered a toxic response. The *S. capricornutum* assay measures the inhibition of photosynthetic activity of an algal culture as a measure of toxicity (Ross, Jarry and Sloterdijk 1988). The rotifer assay is a mortality test (Snell and Personne 1989). The *C. dubia* assay was the standard USEPA (1985) acute assay (48-hour mortality). The dilution water used initially in the toxicity tests and for maintaining the organisms was 1 part Perrier™ bottled water to 9 parts distilled water passed through a Millipore Ultrapure water system. Following a contaminant scare, all Perrier water was removed from the market, so we made our own systhetic dilution water, starting with water from the Ultrapure system and adding salts (Marking and Dawson 1973).

Results of the *C. dubia* bioassay are expressed in toxicity units, as well as 48-hour LC50s, where toxicity units = 100/(48-hour LC50). The 48-hour LC50 is the percent dilution of porewater (or treated porewater) that kills 50% of the test organisms in 48 hours. For example, if a 7% solution (by volume) of porewater in dilution water is the LC50 (see site CS307.4, Table 3.1 in the Results Section), then:

7% = 48-hour LC50
100/LC50 = 100/7 = 14.3 toxic units

meaning that the toxicity in the porewater is more than 14 times the lethal dose.

2.10.2 Standardization of Responses. The results of the various assays were standardized for easier comparisons. The treatment results were divided by the control results and then 1 was subtracted from the quotient. A negative value indicates inhibition (toxicity), a positive value indicates stimulation, and 0 indicates no response (no difference

with respect to the control). If we use the fingernail clam filtering bioassay as an example:

T = test response to sample of sediment porewater

C = control response to uncontaminated dilution water

T = 3.4 mg yeast/g clam/hour

C = 6.5 mg yeast/g clam/hour

T/C = 3.4/6.5 = .52

.52 - 1.00 = -.48 A decline of 48% from the control value, a marked inhibition of the filtering ability of the clams.

2.10.3 Test Samples. A battery of bioassays, including the clam filtering bioassay, were used on raw porewater from Pool 19 of the Upper Mississippi River and from sites along the entire length of the Illinois Waterway, but concentrated in the Chicago metropolitan area (Figures 2.1 and 2.2). The purpose of this comparison was to determine whether the various bioassays consistently identified the same upstream-downstream patterns, including "hot spots" of toxicity. In addition, the clam filtering bioassay was compared to the *Ceriodaphnia dubia* bioassay, using treated porewater from a "hot spot" on the Des Plaines River portion of the waterway (DP 277.0). The purpose of the latter test was to determine how well the filtering bioassay performed within the Toxicity Identification Evaluation (TIE) protocols developed for use with *C. dubia* and other standard reference species at the USEPA's National Effluent Toxicity Assessment Center (NETAC 1989a; Mount 1988; Mount and Anderson-Carnahan 1988 and 1989).

The goal of TIE is to separate toxicants from nontoxic compounds, using sample fractionation techniques in combination with bioassays to determine which fractions contain most of the toxicity. The TIE approach consists of three phases outlined in Figure 2.4. Only samples from Phase 1 treatments were assessed with the clam filtering bioassay. Phase 1 characterizes the physical and chemical properties of the sample toxicants by altering or rendering biologically unavailable generic classes of compounds (Mount and Anderson-Carnahan 1988). After Phase I the toxicants are classified as having characteristics of cationic metals, non-polar organics, volatiles, oxidants, or substances not affected by Phase I methods. The Phase I treatments are outlined in Figure 2.5. The primary tool of Phase I is manipulation of sample pH. The questions asked are: (1) Is toxicity different at different pHs? (2) Does sample manipulation at different pHs affect toxicity? (3) Is toxicity attributable to cationic metals, such as copper or lead? (4) Is toxicity associated with oxidizing agents, such as chlorine or chloramines? The graduated pH test answers the first question and is designed to indicate a pH-dependent toxicant such as un-ionized ammonia. The second question is answered by performing the following tests at different pHs: aeration, filtration and reverse-phase, solid phase extraction (SPE) on $C_{18}$ columns. Aeration tests determine whether toxicity is attributable to volatile or oxidizable compounds. The filtration tests indicate whether

# TOXICITY-BASED TOXICITY IDENTIFICATION EVALUATION

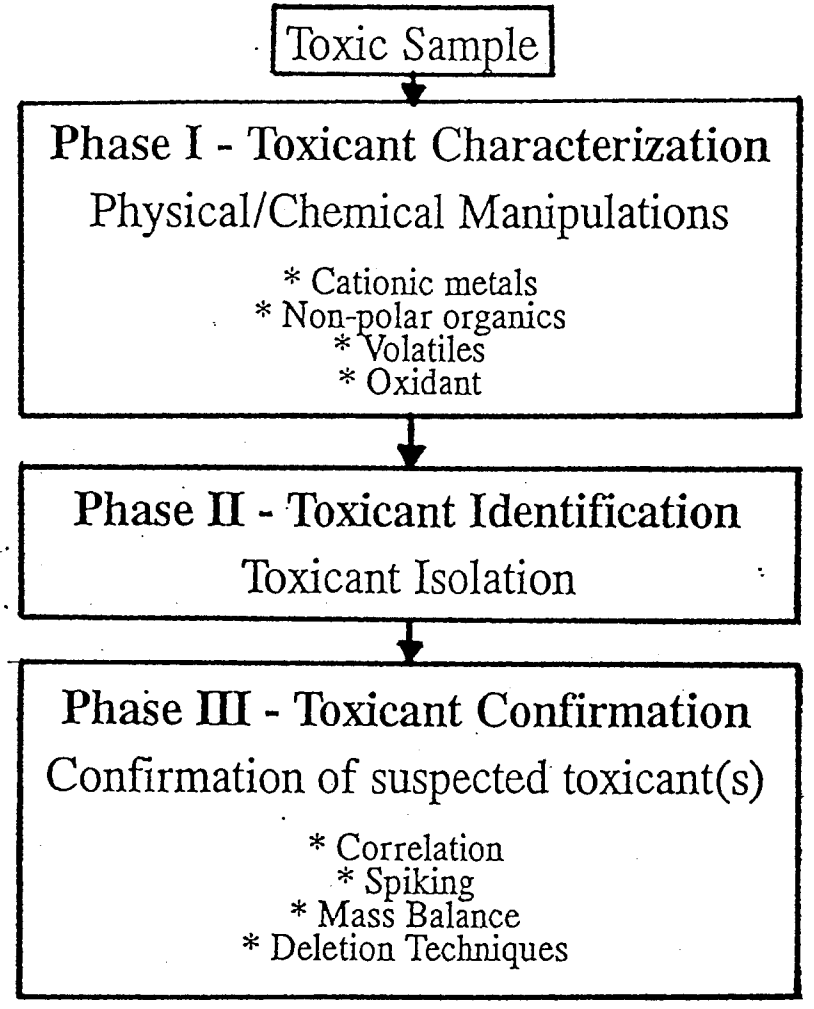


Figure 2.4. The three phases of Toxicity Identification and Evaluation (TIE) procedures.

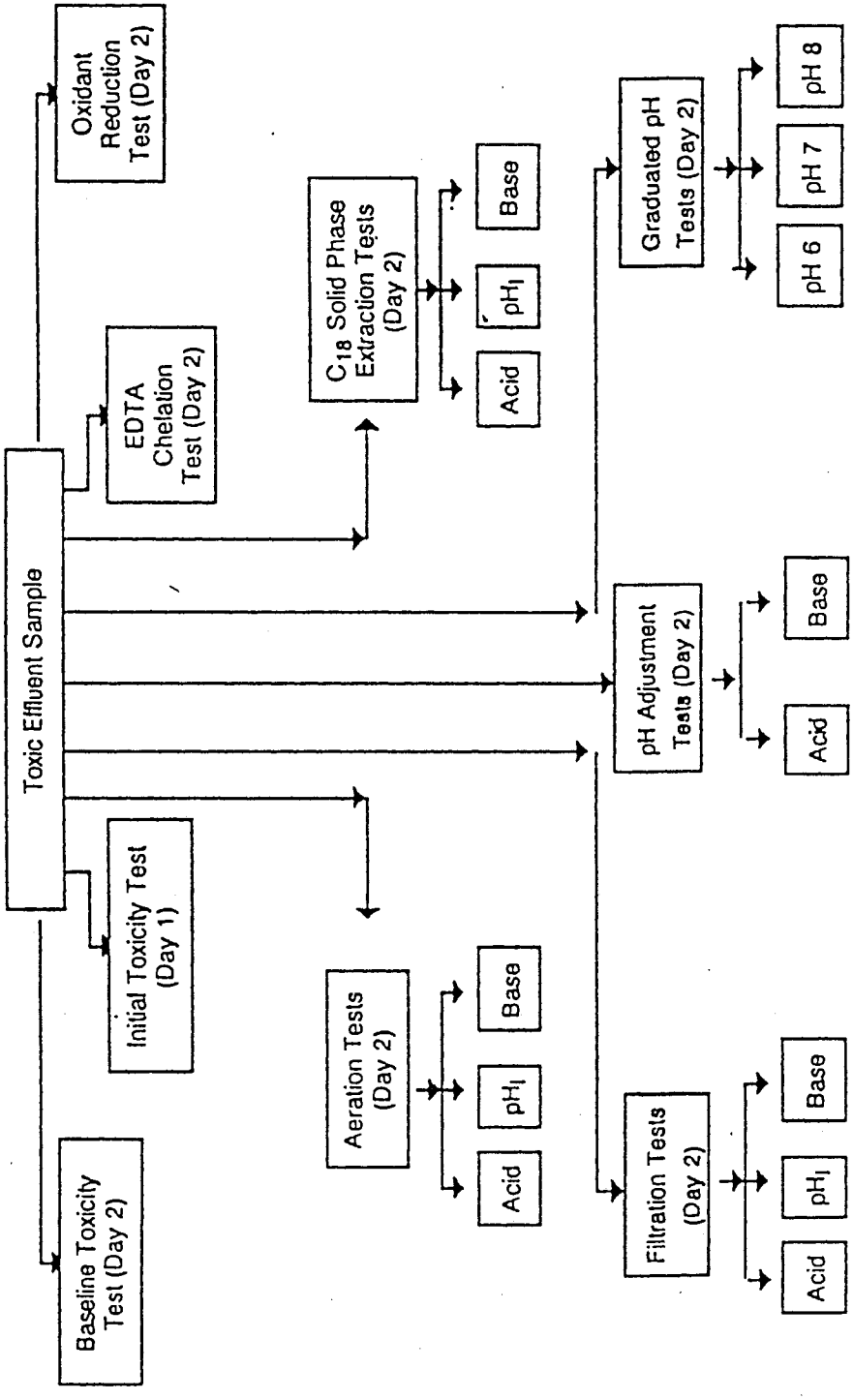


Figure 2.5. Steps involved in Phase I Toxicity Identification and Evaluation (TIE).
Source: Mount and Anderson-Carnahan 1988.

toxicity is associated with filterable components. Reverse-phase SPE indicates whether toxicity is attributable to non-polar compounds. Presence of toxic cationic metals is indicated if addition of a chelating agent, ethylenediaminetetraacetic acid (EDTA), diminishes toxicity. Presence of chlorine or other oxidizing agents is indicated by a reduction in toxicity following addition of the reducing agent, sodium thiosulfate.

# 3.0 RESULTS

## 3.1 Development of the Fingernail Clam Filtering Bioassay

The filtering performance bioassay certainly required less specialized equipment and skill than the clam gill bioassays used previously (Blodgett et al. 1984; Anderson, Sparks, and Paparo 1978; Sparks, Sandusky and Paparo 1981; Sparks, Sandusky and Paparo 1983). As we had hoped, the inhibition of filtering rate was proportional to the amount of the reference toxicant, sodium cyanide, added to test solutions (Figure 3.1). We also observed that some individual fingernail clams would eject water while they were being blotted dry. If some individuals retained water within their valves when they were weighed, and others ejected it, the weight wets and hence the filtering rates per gram wet weight would be highly variable, perhaps masking real differences in response. Another problem was that our clams came from populations that were clearly under various degrees of stress from 1988 to 1991. Many organisms lose tissue mass under stress, which means that the weight of living tissue in our clams might have been below average or highly variable, while the weight of the shells and the water they enclosed would remain the same. Since it is the living tissue that does the filtering, it would be better to measure filtering rates per unit weight of tissue, preferably oven-dried ($100^{0}$ C) tissue, to avoid any additional variation caused by differences in water retention of the tissue.

## 3.2 Comparison with Other Bioassays.

3.2.1 Filtering Assay Compared to Gill Assay. Both the clam gill assay and the clam filtering assay indicate toxicity in sediments in the upper Illinois Waterway, close to the Chicago area, and much less, or no toxicity in Pool 19 of the Upper Mississippi River (Figure 3.2). The filtering assay indicates no toxicity in the Illinois Waterway below River Mile 248 (Figure 3.2). Unfortunately, no samples below River Mile 286.0 were tested with the gill assay for comparison during this project, although sediment samples taken from Quiver Lake (Illinois River Mile 123.0) in November 1980 and water column samples from the main channel at Havana (Illinois River Mile 119.6) in October 1977 were toxic, as measured by the gill assay (Sparks, Sandusky and Paparo 1983 and Anderson, Sparks, and Paparo 1978). Both assays agree that toxicities in the North Branch of the Chicago River (miles 325 and 326) are lower than at some downstream stations, indicating a likely source of toxicity between mile 325 and the downstream points.

The toxicities recorded by the two methods differ in detail, however, which is not too surprising considering that bulk sediment samples collected in March 1989 were used for the gill assay and porewater from samples collected more than two years later, in 1991, were used for the filtering assay. 1988-1989 was a period of drought and extreme low flows in both the Upper Mississippi River and Illinois River, and fingernail clam populations were declining in Pool 19. Flows returned to nearly normal in 1990. The gill assay indicated nearly a 20% inhibition in response to Pool 19 sediments in 1989, whereas the

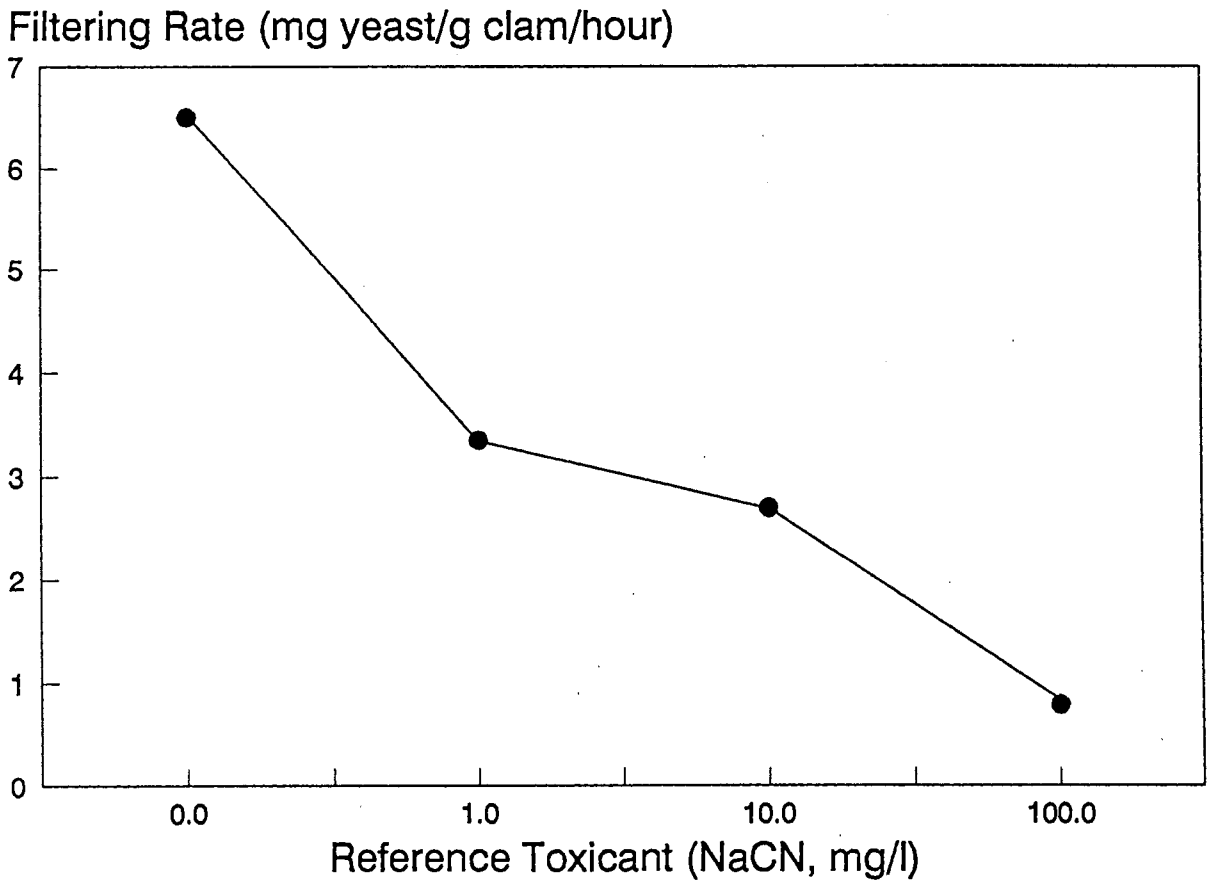


Figure 3.1. Fingernail clam filtering response to a reference toxicant, sodium cyanide (in mg/l added as salt, NaCN).

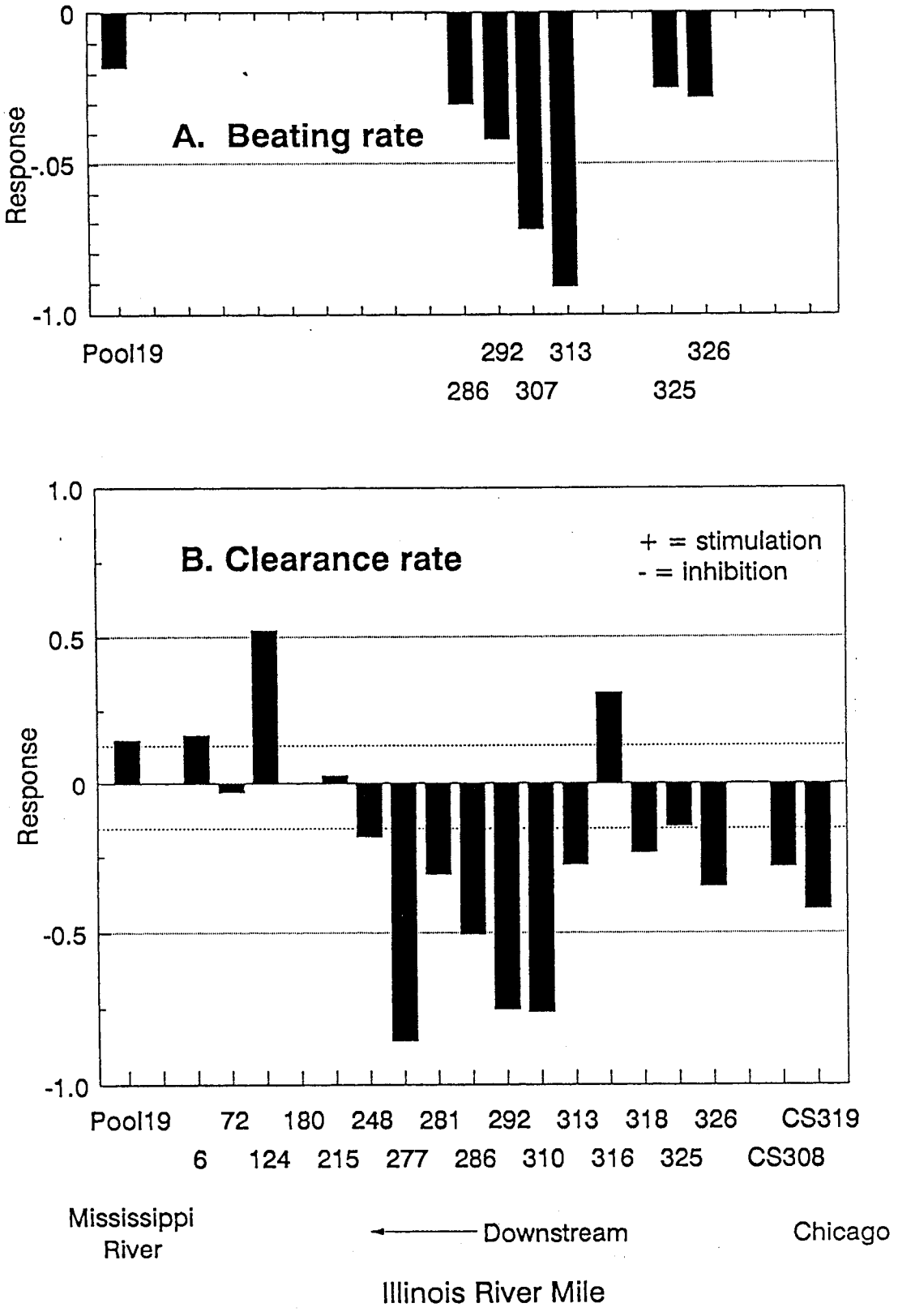


Figure 3.2. Comparison of the response of isolated clam gills (A) and the filtering response of intact clams (B) to sediments from the Illinois Waterway. the gill response is expressed in terms of the inhibition of the beating rate of cilia on the gills, in comparison to control gills maintained in uncontaminated dilution water. The gills were exposed to dilutions of bulk sediments collected in March 1989. The filtering response measures the ability of the clams to clear yeast suspensions, in comparison to controls. These clams were exposed to sediment porewater collected in 1991.

filtering assay indicated a barely significant stimulation in 1991, perhaps in response to sodium, potassium, calcium, and magnesium salts in the porewater. Anderson, Sparks and Paparo (1978) demonstrated the importance of these salts in regulating the beating of the cilia on the gills of the clams. Salts that affect the cilia are likely to affect filtering performance because the lateral cilia produce the water currents that bring food into the clam and the latero-frontal cilia act as filters. Also, the presence of organic matter in the sediment porewaters may have stimulated a feeding response in the clams, which are deposit feeders, as well as water column filterers. The most toxic site on the Illinois Waterway in 1991, according to the filtering assay, was DP 277.0, a site not tested in 1989. The most toxic site in 1989 was SS 313.0, which the filtering assay also rated as toxic in 1991, but less so than 5 sites that were 3-36 miles downstream (Figure 3.2).

**3.2.2 Filtering Assay Compared to Standard Bioassays.** There were marked differences in the responses of the five test organisms to sediment porewater from the same sites (Figure 3.3). Luminescence of the marine bacterium, *Photobacterium phosphoreum*, (Microtox test) was inhibited by 34% at SS313.0 on the Sanitary and Ship Canal and 32% at CS307.4 on the Calumet Sag Channel. Maximum stimulation of approximately 50% occurred at the next site upstream on the Calumet Sag Channel, CS318.5. Responses to porewaters from other sites were slight and variable, sometimes mildly inhibitory and sometimes mildly stimulatory.

Photosynthesis by the freshwater alga, *Selenastrum capricornutum*, was markedly stimulated, by a factor of nearly 2, by sediment porewaters from the mouth of Swan Lake, IR6.0, and the Sanitary and Ship Canal, SS310.0. Stimulation is an indication of nutrient enrichment; e.g., by nitrogen and phosphorus (Ross et al. 1988). The greatest inhibition, -86%, was caused by sediment porewater from Lake Chautauqua, IR125.5, although inhibition also occurred at IR72.0, IR281.1, SS313.0, SS315.3, and CS307.4.

A large percentage of the rotifers, *Branchionus calciflorus*, died in porewaters from Meredosia Lake (IR72) and Lake Chautauqua (IR125.5), but the rotifers exhibited no significant responses anywhere else (Figure 3.3).

In contrast to the microorganisms (bacterium, alga, and rotifer), the macroinvertebrates *C. dubia* and *M. transversum* were remarkably consistent in their responses to the sediment porewaters. Both organisms exhibited no inhibitory response to porewaters from the lower Illinois River or from the reference site in the Upper Mississippi River (Figure 3.3). The clam and the water flea likewise are consistent in indicating toxicity in the upper waterway. Filtering performance in the clam was inhibited starting with sediment porewaters from IR248.2 near Marseilles and water flea mortality started at DP277.0, just above the mouth of the Du Page River near the Interstate 55 bridge.

## 3.3 Pattern of Toxicity in the Illinois Waterway.

Toxicity in sediment porewaters, as measured by bioassays using macroinvertebrates as test organisms, increases in the upstream direction, toward the Chicago-Joliet area, indicating a likely source of toxicity in that region. Sediment porewaters from 7 of the 13 upstream

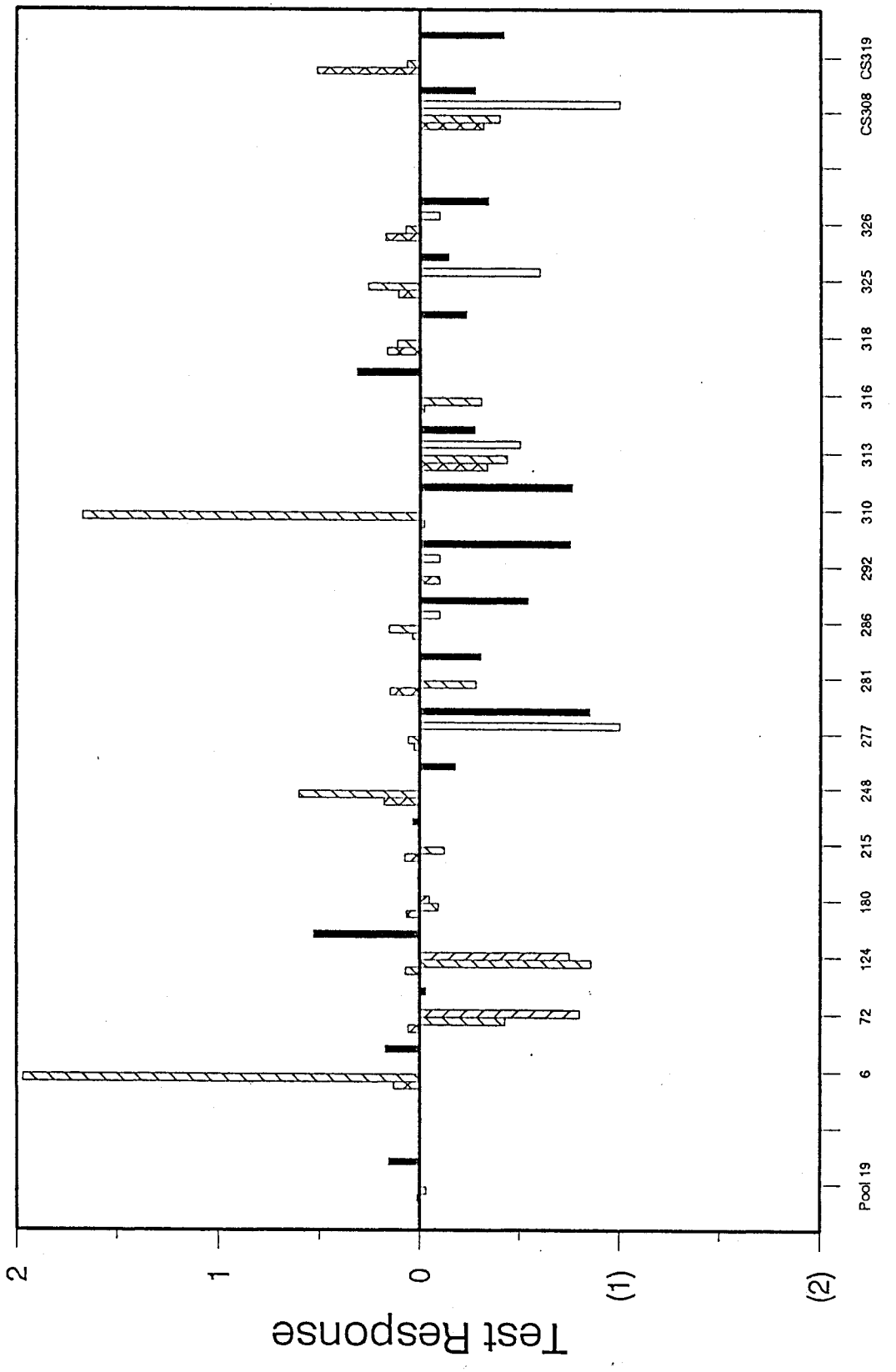


Figure 3.3. Responses of five organisms to porewaters from sediments of the Illinois Waterway and from a reference site (Pool 19 of the Upper Mississippi River). Numbers above zero represent stimulation, numbers below zero represent inhibition (toxicity). CS = Calumet Sag Channel. FNC = fingernail clams.

sites were toxic to the water flea *C. dubia*, and 12 of 13 inhibited the filtering performance of the fingernail clam (Figure 3.3). Neither organism indicated any toxicity in porewaters from the lower Illinois River or in Pool 19 of the Upper Mississippi River. The toxicity of porewater to *C. dubia* appeared to be closely related to the total ammonia concentration in the porewater in 1989 and 1990, with the exception of site DP277.0 in 1990, where factors other than ammonia may have contributed to the peak toxicity there (Figure 3.4). Toxicity to *C. dubia* correlated with total ammonia in porewater samples taken in 1990 and 1991 ($r = 0.85$, Figure 3.5). Porewaters generally had much higher total ammonia concentrations and greater conductivities than surface waters from the same sites (Table 3.1).

Porewater from site DP277.0 was most toxic to the fingernail clam, and highly toxic to the water flea, so it was subjected to TIE Phase I analysis, the results of which are described below. This was the only TIE analysis that employed both types of bioassays (*M. transversum* and *C. dubia*) and therefore is included in the results section of this report. All other TIE analyses used *C. dubia* toxicity tests and these results are reported in Sparks, Ross, and Dillon (1992).

## 3.4 Toxicity Identification and Evaluation (TIE).

The *C. dubia* mortality test and the *M. transversum* filtering assay both indicated that toxicity of porewater from DP277.0 was not removed by chelation with EDTA, so toxicity was not attributable to heavy metals (Table 3.2). Both tests were consistent in indicating that toxicity persisted when the bioassays were run at both lower (6.5) and higher (8.5) pHs (Table 3.2). The clam assay indicated toxicity increased at both the higher and lower pH, a result that might be caused by the presence of two toxicants whose chemical equilibria are pH-sensitive. For example, the proportion of ammonia existing in the toxic, un-ionized form ($NH_3$) will increase at higher pHs, and the proportion of hydrogen sulfide existing in the toxic, un-ionized form ($H_2S$) increases at lower pHs. Both bioassay techniques indicated that toxicity decreased from the initial baseline value upon standing or with aeration at the higher pH of 11 (Table 3.2). This result is consistent with the presence of ammonia, whose un-ionized form can be oxidized and volatilized.

Results of the two bioassays are inconsistent with each other in virtually all the other combinations of treatments. According to the *C. dubia* bioassay, virtually all the toxicity was removed by filtration or passage through the $C_{18}$ column at a pH of 3, whereas the clam assay recorded the highest toxicity (-0.94) with the $C_{18}$C-pH 3 treatment and considerable toxicity even after filtering the porewater (Table 3.2). The clam assay recorded the least toxicity (-0.04) following aeration at a pH of 3, whereas there was considerable mortality in the *C. dubia* bioassay.

## 3.5 Ammonia Tolerance of Clams from Different Sources

3.5.1 Field Observations. In October and November 1991 we tried to obtain sufficient clams from Pool 19 of the Mississippi River, Swan Lake on the Illinois River, and from waterways in the Chicago area to assay ammonia tolerance of clams from populations that presumably had been exposed to different levels of ammonia in sediment pore water.

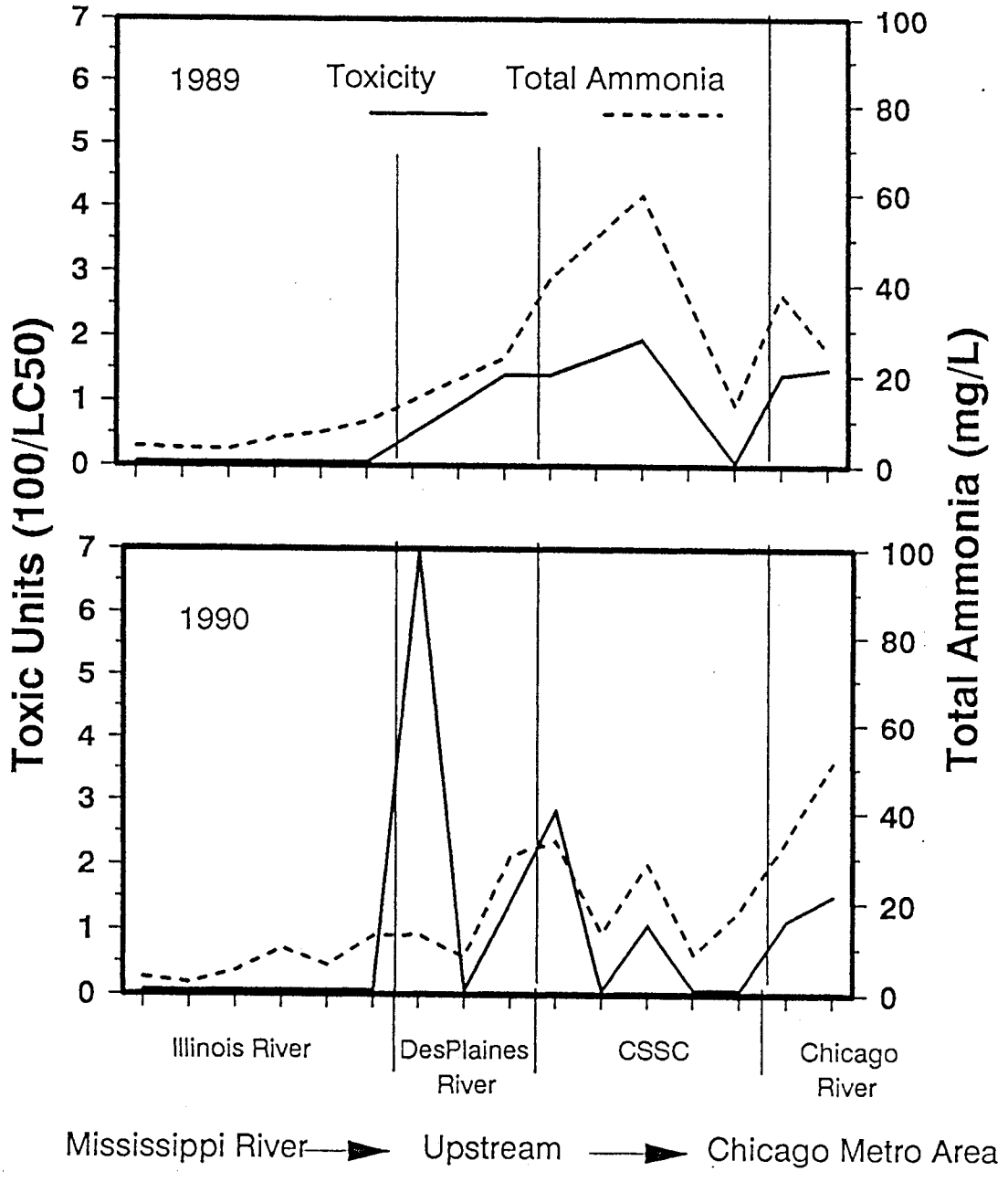


Figure 3.4. Toxicity and total ammonia concentrations in sediments of the Illinois Waterway in 1989 and 1990. Toxicity was measured with the *Ceriodaphnia dubia* bioassay. The highest toxicity observed (in the Des Plaines River portion of the Waterway, DP277.0, in 1990) was attributed to a combination of ammonia and petroleum-related hydrocarbons.

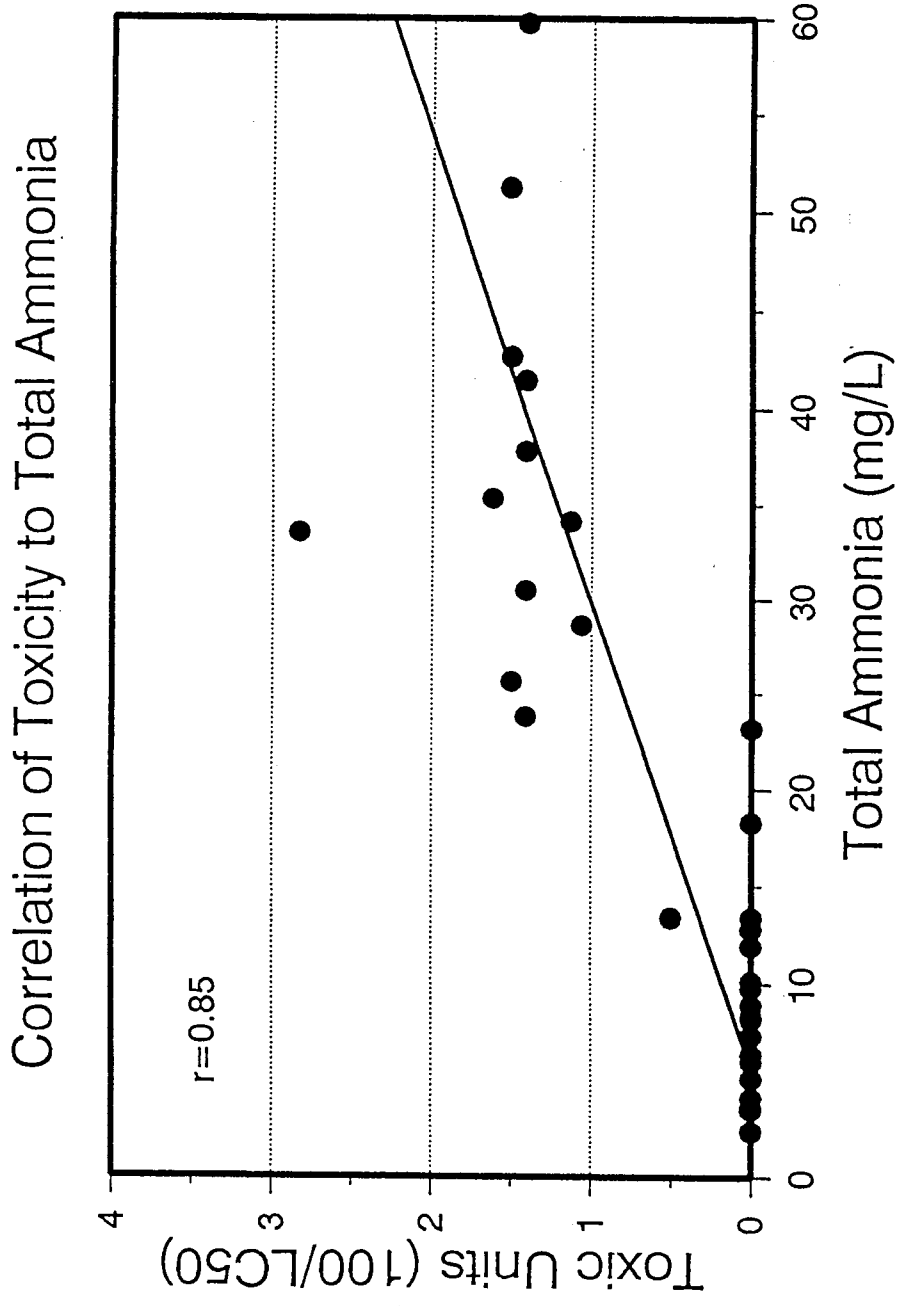


Figure 3.5. Correlation of toxicity with total ammonia concentrations (mg/l ammonia as nitrogen) in sediment porewaters. Toxicity is expressed in toxic units, 100/LC50, where LC50 is the dilution of porewater in clean water that causes half of the Ceriodaphnia dubia to die within 48 hours.

Table 3.1. Characteristics of surface water (S) and sediment pore water (P) from sites on the Illinois Waterway and the Upper Mississippi River.

| Station No. (River Mile) | | Temp °C | D.O. mg/L | pH | Cond. lmhos/cm | Hardness mg $CaCO_3$/L | Alk. mg $CaCO_3$/L | Total $NH_3$-N mg/L | Un-ionized $NH_3$-N mg/L | Chlorine mg/L | $H_2S$ mg/L | $S^{2-}$ mg/l | TOC % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CR326.4 | P | 23.6 | 3.20 | 7.17 | 1395 | 488 | 568 | 42.0 | 0.3167 | 0.10 | 0.000 | 0.020 | 7.15 |
| | S | 24.6 | 9.00 | 7.28 | 859 | --- | --- | 2.5 | 0.0260 | ---- | ----- | ----- | ---- |
| CR324.8 | P | 23.1 | 4.80 | 7.08 | 1071 | 270 | 402 | 28.0 | 0.1659 | 0.10 | 0.000 | 0.010 | 6.19 |
| | S | 23.0 | 9.10 | 7.43 | 661 | --- | --- | 1.6 | 0.0209 | ---- | ----- | ----- | ---- |
| CS318.5 | P | 23.7 | 6.20 | 7.13 | 1533 | 684 | 422 | 11.0 | 0.0762 | 0.05 | 0.000 | 0.020 | 6.27 |
| | S | 20.6 | 8.20 | 7.27 | 1071 | --- | --- | 4.0 | 0.0306 | ---- | ----- | ----- | ---- |
| CS307.4 | P | 24.0 | 5.80 | 6.53 | 1510 | 540 | 506 | 41.0 | 0.0733 | 0.08 | 0.000 | 0.020 | 4.03 |
| | S | 22.7 | 7.20 | 6.97 | 571 | --- | --- | 1.9 | 0.0085 | ---- | ----- | ----- | ---- |
| SS317.0 | P | 22.5 | 5.80 | 7.22 | 798 | 312 | 304 | 15.0 | 0.1173 | 0.03 | 0.000 | 0.015 | 7.12 |
| | S | 23.1 | 7.50 | 7.15 | 450 | --- | --- | 1.6 | 0.0111 | ---- | ----- | ----- | ---- |
| SS315.3 | P | 21.0 | 1.90 | 6.79 | 631 | 254 | 208 | 7.3 | 0.0191 | 0.08 | 0.000 | 0.050 | 3.23 |
| | S | 20.7 | 8.30 | 7.13 | 469 | --- | --- | 0.8 | 0.0045 | ---- | ----- | ----- | ---- |
| SS313.0 | P | 24.2 | 0.70 | 6.85 | 922 | 211 | 336 | 19.0 | 0.0718 | 0.02 | 0.000 | 0.070 | 4.33 |
| | S | 26.0 | 7.60 | 7.16 | 532 | --- | --- | 0.6 | 0.0052 | ---- | ----- | ----- | ---- |
| SS310.0 | P | 27.0 | 4.60 | 7.04 | 740 | 258 | 216 | 11.0 | 0.0782 | 0.00 | 0.000 | 0.050 | 0.93 |
| | S | 26.5 | 8.40 | 6.82 | 686 | --- | --- | 2.2 | 0.0091 | ---- | ----- | ----- | ---- |
| SS292.2 | P | 19.9 | 3.40 | 7.32 | 1172 | 384 | 390 | 27.5 | 0.2241 | 0.05 | 0.000 | 0.070 | 6.77 |
| | S | 22.1 | 9.40 | 7.32 | 1006 | --- | --- | 4.6 | 0.0439 | ---- | ----- | ----- | ---- |
| DP286.3 | P | 23.3 | 3.20 | 7.06 | 1262 | 484 | 478 | 25.0 | 0.1435 | ---- | ----- | 0.080 | 9.42 |
| | S | 26.7 | 8.60 | 7.56 | 1180 | --- | --- | 1.9 | 0.0431 | ---- | ----- | ----- | ---- |
| DP281.1 | P | 22.0 | 8.00 | 7.20 | 891 | 468 | 412 | 6.7 | 0.0483 | 0.05 | 0.000 | 0.038 | 7.63 |
| | S | 23.2 | 9.60 | 7.70 | 608 | --- | --- | 0.9 | 0.0220 | ---- | ----- | ----- | ---- |
| DP277.0 | P | 25.1 | 7.40 | 7.84 | 1366 | 646 | 580 | 10.8 | 0.4108 | 0.27 | 0.000 | 0.270 | 3.91 |
| | S | 25.4 | 9.00 | 8.18 | 671 | --- | --- | 0.6 | 0.0487 | ---- | ----- | ----- | ---- |
| IR248.2 | P | 26.1 | 4.20 | 7.19 | 977 | 382 | 394 | 9.5 | 0.0894 | 0.02 | 0.000 | 0.040 | 3.17 |
| | S | 25.1 | 8.00 | 7.59 | 424 | --- | --- | 2.0 | 0.0435 | ---- | ----- | ----- | ---- |
| IR215.0 | P | 21.5 | 5.60 | 7.48 | 674 | 306 | 257 | 5.2 | 0.0685 | 0.025 | 0.000 | 0.030 | 3.13 |
| | S | 20.9 | 7.90 | 7.31 | 537 | --- | --- | 1.2 | 0.0103 | ---- | ----- | ----- | ---- |

Table 3.1. cont.

| Station No. (River Mile) | | Temp °C | D.O. mg/L | pH | Cond. Imhos/cm | Hardness mg $CaCO_3$/L | Alk. mg $CaCO_3$/L | Total $NH_3$-N mg/L | Un-ionized $NH_3$-N mg/L | Chlorine mg/L | $H_2S$ mg/L | $S^{2-}$ mg/l | TOC % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IR180.0 | P | 21.4 | 6.40 | 7.55 | 826 | 346 | 344 | 8.3 | 0.1272 | ---- | ----- | 0.040 | 2.34 |
| | S | 21.2 | 8.80 | 7.55 | 550 | --- | --- | 1.5 | 0.0227 | 0.05 | 0.000 | ----- | ---- |
| IR125.5 | P | 22.7 | 6.90 | 7.41 | 540 | 292 | 278 | 4.2 | 0.0514 | 0.00 | 0.000 | 0.180 | 2.90 |
| | S | 21.5 | 9.30 | 7.05 | 559 | --- | --- | 0.9 | 0.0044 | ---- | ----- | ----- | ---- |
| IR 72.0 | P | 22.3 | 7.20 | 7.34 | 682 | 352 | 318 | 2.0 | 0.0203 | 0.15 | 0.000 | 0.010 | 2.39 |
| | S | 20.3 | 9.40 | 7.63 | 498 | --- | --- | 0.4 | 0.0068 | ---- | ----- | ----- | ---- |
| IR 6.0 | P | 25.0 | 4.80 | 7.05 | 757 | 272 | 230 | 3.0 | 0.0190 | 0.00 | 0.000 | 0.150 | 0.75 |
| | S | 19.6 | ---- | 7.84 | 553 | --- | --- | 0.5 | 0.0130 | ---- | ----- | ----- | ---- |
| MR377.0 | P | 27.9 | 5.00 | 7.10 | 1011 | 500 | 460 | 14.0 | 0.1215 | 0.00 | 0.000 | 0.064 | 1.83 |
| | S | 25.7 | ---- | 7.19 | 354 | --- | --- | 0.8 | 0.0073 | ---- | ----- | ----- | ---- |

Note: 1. mg/l is the same as ppm

2. To convert temperature in °C to °F, multiply by 9/5 and add 32.

Table 3.2. Comparison of responses of fingernail clams[a] (*Musculium transversum*) and water fleas[b] (*Ceriodaphnia dubia*) to sediment pore water from the Des Plaines River portion of the Illinois Waterway (DP277.0). The pore water was subjected to various treatments to remove or change the toxicity of suspected contaminants.

| Treatment | pH 3 | pH Initial | pH 11 |
|---|---|---|---|
| 1. Standing | | | |
| *C. dubia* | 6.2 | 13.8[c] | 5.6 |
| *M. transversum* | -0.139 | -0.328 | -0.202 |
| 2. Aerated | | | |
| *C. dubia* | 9.9 | 11.3 | 6.2 |
| *M. transversum* | -0.045 | -0.431 | -0.187 |
| 3. Filtered | | | |
| *C. dubia* | 0 | 2.8 | 1.12 |
| *M. transversum* | -0.457 | -0.795 | -0.352 |
| 4. $C_{18}$ | | | |
| *C. dubia* | 0 | 1.59 | 1.4 |
| *M. transversum* | -0.943 | -0.198 | -0.328 |
| | | pH | |
| 5. Graduated pH | 6.5 | 7.5 | 8.5 |
| *C. dubia* | Toxic | Toxic | Toxic |
| *M. transversum* | -0.513 | -0.353 | -0.405 |
| | | pH | |
| 6. EDTA | | Initial | |
| *C. dubia* | | Toxic | |
| *M. transversum* | | -0.636 | |

Notes: [a]The *M. transversum* response is the decline in filtering rate, following 1 hour of exposure to porewater, relative to the control filtering rate. -1.000 = complete cessation of filtering. 0.000 = no reduction.

[b]The *C. dubia* response is measured in toxic units = 100/(48-hour LC50). The LC50 is the percent dilution of porewater (or treated porewater) that kills 50% of the water fleas in 48 hours.

[c]The baseline toxicity of the porewater, prior to any treatment: i.e., the porewater is 13.8 times the lethal level for the water flea and depresses the filtering rate of the clam by 32.8%.

In the first 4 treatments the pH of the porewater is adjusted to the values shown and treated. Then the pH is readjusted to the initial value (7.8) and bioassayed. The porewater is allowed to stand, aerated, filtered, or subjected to reverse-phase, solid phase extraction on a $C_{18}$ column.

In treatment 5, the pH is adjusted to the values shown, and bioassayed.

In treatment 6, the porewater is bioassayed following treatment with the chelating agent EDTA to tie up heavy metals.

However, several sites on the Sanitary and Ship Canal (SS 319.3, SS 312.7, and SS 310.0) where we had obtained clams two months previously (July) now contained few or no live clams, but many recently dead shells, indicating that inimical conditions had developed in the interim (Table 3.3). Another indication of stress was the relatively small size of clams in some areas, such as the Sanitary and Ship Canal at Lockport (SS 292.7, Table 3.3). A normal population in the fall of the year should contain some very small newborns (1-2 mm shell length) and some adults up to at least 12 mm in shell length. Also of note was the presence of the European zebra mussel in the Sanitary and Ship Canal (SS 312.7).

We were successful in obtaining sufficient clams from Pool 19 of the Upper Mississippi River, Swan Lake of the Illinois River, and from two of the waterways in Chicago (the Sanitary and Ship Canal and the Calumet Sag Channel) to run the comparative assays, described next.

3.5.2 Comparative Bioassays. Clams from the six sources differed greatly in the baseline filtering rates of the control animals that were not exposed to added ammonia (Table 3.4). The control rates differed markedly among clams taken from different sites within the same locale; e.g., the control rate for clams from Swan Lake site 1 was nearly double that from site 2 (6.69 vs 3.53 mg yeast/mg clam tissue) and the rate from site 1 of the Sanitary and Ship Canal at Lockport was two thirds that from site 2 (4.15 vs. 6.14). Excluding these two values of 3.53 and 4.15 mg yeast/mg clam, the control rates averaged 6.58 $\pm$ standard deviation of 0.54 (range 6.14 - 7.31). The low control rates may indicate the clams had been subject to some kind of stress at those two sites. Since one site is from the upper waterway (Lockport) and the other from the extreme lower portion of the waterway (Swan Lake), these results indicate that populations throughout the waterway may be under stress, at least in certain local microhabitats.

The experimental groups of clams from the various sources were challenged by exposure to added ammonia. We expected that clams that had been chronically exposed to ammonia might have undergone selection for ammonia tolerance and thus exhibit less response to added ammonia. The clams with the lowest control filtering rate (Swan Lake site 2) actually increased their rate in response to added ammonia, but never came within the control range (Table 3.4). The clams from Swan Lake site 1 did not decrease their filtering significantly (the criterion for a significant response is a change greater than 10.6% from the control rate), but the highest un-ionized ammonia nitrogen concentration achieved in this test (0.0858 mg/l) was lower than the highest concentrations in all of the other tests (0.0943 - 0.2517 mg/l, Table 3.4).

The filtering rate of clams from the Calumet Sag Channel near Chicago increased slightly, but the increase in just one concentration exceeded the response criterion (maximum change was +10.7%). Clams from all other sources decreased their filtering rates significantly when challenged with ammonia. In summary, clams from Swan Lake site 2 appeared to have been under some previous stress (based on their exceptionally low baseline filtering rate) and to be relatively insensitive to ammonia, based on the increase in their filtering rates above baseline in response to added ammonia. Clams from Swan Lake site 1 and from the Calumet Sag Channel likewise showed little response to added ammonia, whereas clams from both sites at Lockport (in the Sanitary and Ship Canal) and from Pool 19 of the Upper Mississippi River

**Table 3.3.** Qualitative observations on the status of clam populations in Chicago waterways, Pool 19 of the Upper Mississippi River, and Swan Lake of the Illinois River in October and November 1991. Duration of sampling or number of samples is noted.

| Site, Date | Qualitative Observations (Mt = *Musculium transversum* Ss = *Sphaerium striatinum*) |
|---|---|
| MR 365.5 Pool 19, Miss. R. 11 Oct 1991 | 300 Mt in 2 hr, ranging from newborns to 12 mm. Most were 5-7 mm. |
| CR 326.4 N. Branch, Chicago R. 29 Oct 1991 | 150-200 Ss in 2 Ekman grab samples. |
| CS 318.5 Cal-Sag Channel 29 Oct 1991 | 200-250 Mt in 4 Ekman samples. |
| SS 319.3 SS Canal 4 Oct 1991 | No live clams in several hours. Substrate consisted mostly of dead clam shells, 10-15% recently dead. Live clams had been obtained in July. |
| SS 312.7 SS Canal 4 Oct 1991 | No native clams, just 15 small zebra mussels in 15 min. |
| SS 310.0 SS Canal 4 Oct 1991 | No live clams. |
| SS 292.7 SS Canal, Lockport 4 Oct 1991 | 25 Mt and 25 Ss in about 50 min. No Mt larger than 7 mm. Ss were 6-10 mm. |
| SS 292.7 SS Canal, Lockport 12 Nov 1991 | 300 Mt and 75-100 Ss in several hours. |
| IR 5.1 Swan Lake, Il. R. 17 Oct 1991 | 325 Mt in 2 hr. 20-30% under 5 mm, 50% 7-9 mm, 10-15% 9-11 mm, 10-15% 11-14 mm. |

Table 3.4 Response of fingernail clams (*Musculium transversum*) from different sources to ammonia.

| Source of Clams | $NH_3$-N (mg/l)[a] Initial | Final | Mean | Filtering Rate mg yeast/mg clam[b]/h | Percent Change from Control |
|---|---|---|---|---|---|
| Pool 19 | 0.0023 | 0.0205 | 0.0114 | 7.31 | Control |
| MR 365.5 | 0.0122 | 0.0367 | 0.0245 | 5.20 | -28.9% |
| | 0.0230 | 0.0275 | 0.0253 | 2.52 | -65.5% |
| | 0.1168 | 0.1465 | 0.1317 | 1.95 | -73.3% |
| Blue Island | 0.0008 | 0.0037 | 0.0023 | 6.19 | Control |
| CS 318.5 | 0.0152 | 0.0158 | 0.0155 | 6.52 | 5.3% |
| | 0.0233 | 0.0180 | 0.0207 | 6.85 | 10.7% |
| | 0.1312 | 0.0573 | 0.0943 | 6.78 | 9.5% |
| Lockport | 0.0015 | 0.0039 | 0.0027 | 4.15 | Control |
| SS 292.7 | 0.0132 | 0.0196 | 0.0164 | 2.10 | -49.4% |
| Site 1 | 0.0188 | 0.0239 | 0.0214 | 1.67 | -59.8% |
| | 0.0991 | 0.1420 | 0.1206 | 1.63 | -60.7% |
| Lockport | 0.0016 | 0.0021 | 0.0019 | 6.14 | Control |
| SS 292.7 | 0.0279 | 0.0402 | 0.0341 | 5.92 | -3.6% |
| Site 2 | 0.0625 | 0.0702 | 0.0664 | 3.36 | -45.3% |
| | 0.2535 | 0.2499 | 0.2517 | 1.34 | -78.2% |
| Swan Lake | 0.0028 | 0.0053 | 0.0041 | 6.69 | Control |
| IR 5.1 | 0.0106 | 0.0252 | 0.0179 | 6.18 | -7.6% |
| Site 1 | 0.0195 | 0.0256 | 0.0226 | 6.68 | -0.1% |
| | 0.0955 | 0.0760 | 0.0858 | 6.00 | -10.3% |
| Swan Lake | 0.0032 | 0.0035 | 0.0034 | 3.53 | Control |
| IR 5.1 | 0.0177 | 0.0213 | 0.0195 | 4.66 | 32.0% |
| Site 2 | 0.0256 | 0.0305 | 0.0281 | 5.84 | 65.4% |
| | 0.0850 | 0.1045 | 0.0948 | 5.86 | 66.0% |

[a] Concentration is expressed as un-ionized ammonia nitrogen
[b] Dry tissue weight, minus shell

decreased their filtering rates substantially in response to ammonia. Clams from different sources do vary in their tolerance of ammonia, but their tolerance does not increase in the upstream direction on the Illinois Waterway, where ammonia concentrations in sediment pore water tend to be higher than in downstream areas.

# 4.0 DISCUSSION

## 4.1 The Fingernail Clam Filtering Bioassay

The fingernail clam filtering bioassay can be performed with relatively simple equipment commonly available in most laboratories. It is relatively quick, requiring only one hour of exposure to the test solution, followed by one hour to assess filtering performance. It measures a sublethal response, filtering ability, that is critical to the survival of the organism, so the bioassay meets the test of physiological relevance to the organism. The bioassay also meets the test of ecological relevance because the test species, *M. transversum*, is an important link in food chains leading to fish and ducks and it is a dominant member of the benthic macroinvertebrate community in many Midwestern aquatic systems. Another aspect of ecological relevance is that *M. transversum* is a member of the infauna, the organisms that burrow into the sediment rather than living on top of the sediment or on plants, snags or other structure extending into the water column, so it is actually exposed in the environment to the sediment porewater we were testing. In contrast, many of the standard test organisms, including *C. dubia*, are water column dwellers or structure dwellers that never come in contact with sediment porewater in nature. The filtering response is graded; i.e., the degree of inhibition of filtering performance is a function of the concentration of the toxicant, as demonstrated in the test with a reference toxicant, sodium cyanide.

The results obtained by the two types of clam bioassays were generally consistent. In one bioassay, the beating rate of cilia on isolated clam gills was inhibited by bulk sediments from the upper Illinois Waterway, even though the sediments were diluted with molluscan saline solution. In the other bioassay, the filtering rate of intact clams was inhibited by sediment porewaters from the same area. The same porewaters caused significant mortality in *C. dubia*. In contrast, porewaters from some of the same sites stimulated two other standard test organisms, the freshwater alga *Selenastrum capricornutum* and the marine bacterium *Photobacterium phosphoreum* (Microtox™ test). In view of the great physiological differences among plants, bacteria and animals this result is not too surprising; e.g., ammonia is toxic to aquatic animals at relatively low concentrations, but is used as a nitrogen source by plants and some bacteria.

## 4.2 Toxicity in the Illinois Waterway

Two different patterns of toxicity apparently occur in the sediment porewaters of the Illinois Waterway. There is a gradient of increasing toxicity in the upstream direction, associated with increasing concentrations of total ammonia in the sediments (Figures 3.2, 3.4, and 3.5). The second pattern is characterized by patches of toxicity associated with polycyclic aromatic hydrocarbons (PAHs), such as naphthalene, and long-chain hydrocarbons, both evidently derived from petroleum (Sparks, Ross and Dillon 1992). One of the latter sites was located on the lower Des Plaines River section of the waterway, near several refineries. Previous studies have measured elevated levels of metals, pesticides, PAHs, and PCBs in the sediments of the upper

Illinois Waterway (IEPA 1990) and demonstrated that sediments are toxic (Sparks, Sandusky and Paparo 1981; Blodgett et al. 1984; Schubauer-Berigan and Ankley 1991). The two toxicity problems might even be related: Ankley et. al. (1991) suggest that natural microbial processes in aquatic ecosystems may be compromised by organic loading or selective toxicity. The alteration of microbial processes could play a role in the incidence of ammonia accumulation and subsequent toxicity in sediments in the Upper Illinois Waterway.

It is well established that certain sediments can contain high concentrations of ammonia (Keeney 1973, Berner 1980). Nitrogen-containing organic matter is decomposed in sediments by heterotrophic bacteria. The amount of ammonification that takes place depends on oxygen availability (Kleerekoper 1953). Ammonia can accumulate to toxic levels under anaerobic conditions (Berner 1980). Ammonia formation is greatest about 10 cm below the sediment-water interface (Serruya 1974). In this situation, ammonia probably diffuses from the deeper sediments to surficial sediments, and perhaps even to the overlying water, especially if sediments are resuspended by currents or boat- or wind-driven waves. The fingernail clam, *Musculium transversum*, the organism of primary interest in this study, makes shallow burrows in the sediment and may be exposed to much higher levels of ammonia than organisms living in the water column.

*M. transversum* is sensitive to ammonia. Anderson, Sparks and Paparo (1978) found that un-ionized ammonia concentrations of 0.08-0.09 mg/l inhibited the cilia on the gills of the clams, and the growth of the clams in the laboratory was reduced at concentrations between 0.20 and 0.34 mg/l $NH_3$-N. The *C. dubia* acute LC50 for ammonia is 1.04 mg/l (Ankley et. al. 1990). Arthur et. al (1987) reported un-ionized ammonia toxicity to 5 invertebrates that ranged from 1.95 to 18.3 mg/l un-ionized ammonia, as nitrogen (see below).

| SPECIES | LC50 (mg/l) |
|---|---|
| Snail | |
| *Physa gyrina* - adult | 1.95 |
| *Helisoma trivolvis* - adult | 2.17 |
| Amphipod | |
| *Crangonyx pseudogracilis* - adult | 3.12 |
| Mayfly | |
| *Callibaetis skokianus* - nymph | 3.12 |
| Isopod | |
| *Asellus racovitzai* - adult | 5.02 |
| Caddisfly | |
| *Philarctus giaeris* - larvae | 10.1 |
| Crayfish | |
| *Orconectes immunis* - adult | 18.3 |

Concentrations of un-ionized $NH_3$-N ranged up to 0.41 in the sediments in the Upper Illinois Waterway, based on total ammonia concentrations and pHs measured in samples returned to the laboratory (Table 3.1)--sufficient to impair filtering and reduce growth. Ammonia places organisms in double jeopardy because it exerts an oxygen demand in the process of nitrification (conversion to nitrites and then nitrates) and low oxygen levels place organisms under additional stress (USEPA 1985). Ammonification may be occurring in the deep, anaerobic zones of the sediments and nitrification in the shallower, aerobic zones, or in the boundary water at the sediment surface, so benthic invertebrates are exposed to the worst of both worlds: they are exposed to ammonia and to low oxygen at the same time.

The highest ammonia concentrations in sediments are associated with nitrogen-enriched sediments or high organic loading, as from sewage treatment plants (Brezonik 1973; Ankley et. al. 1990; and Schubauer-Berigan and Ankley 1991). Although most sewage treatment plants remove a substantial portion of carbon that is in municipal waste, most do not remove nitrogen, but convert it from ammonia into nitrate. It is possible that nitrate is carried down into the sediments where it is converted back into ammonia in the anaerobic zones. If this is the case, ammonia toxicity in the sediments might be reduced by reducing the nitrogen loading of the river.

The proportion of total ammonia existing in the toxic, un-ionized form is controlled primarily by pH and temperature (Emerson et. al. 1975). The pH of sediments can fluctuate dramatically on a seasonal basis, and the pH of the overlying water can fluctuate daily, so that episodes of toxicity may occur even if the total ammonia concentration remains relatively constant. Ammonia loading of rivers tends to increase during winter because the microorganism-mediated conversion of ammonia to nitrate stops at cold temperatures. Also, aquatic vegetation does not remove ammonia (a plant nutrient) during winter dormancy. Water quality standards frequently allow higher levels of ammonia in the winter because the proportion of total ammonia existing in the toxic, un-ionized form is less at cold temperatures. However, the sensitivity of fish to ammonia increases at cold temperatures, and this biological effect overrides the physical-chemical effect of cold temperature on ammonia equilibria (Reinbold and Pescitelli 1990). Research is needed to determine the effect of cold temperatures on the sensitivity of invertebrates, such as fingernail clams, as well as fish, to ammonia.

During the course of this study, several species of fingernail clams, including *M. transversum*, reappeared in the Chicago area waterways and in the Illinois River at Peoria and Havana. There are at least four possible explanations for this surprising reappearance of clams in the same general areas where the porewaters tested toxic. First, we found that clams from different locations differ in their sensitivity to ammonia, and clams from at least one site on the upper Illinois (the Calumet Sag Channel) responded less to added ammonia than the clams from Pool 19 and from one site in Swan Lake on the lower Illinois, where the organisms were obtained for all of the early bioassays. Second, our previous research demonstrated that the surface layers of sediment in some areas are less toxic than layers a few centimeters deeper (Sparks, Sandusky and Paparo 1981; Blodgett et al. 1984). Toxicity may have been overestimated in tests where surface and deep layers of sediment were mixed prior to testing. Third, toxic

episodes may be brief and infrequent, allowing organisms to colonize in between episodes. In the span of just two months, we sometimes found live clams in the Sanitary and Ship Canal where there had been none before, and conversely, recently-dead shells where live clams had been relatively abundant (see section 3.5.1 and Table 3.3). Fourth, the distribution of toxicity in sediments may be extremely patchy, so that healthy organisms are found adjacent to barren or stressed areas. Clams from adjacent sites in both Swan Lake on the lower Illinois River and at Lockport in the Chicago Sanitary and Ship Canal differed markedly in their control filtering rates and in their sensitivity to added ammonia, indicating that they had experienced different levels of stress prior to collection (Table 3.4). If the latter two hypotheses prove to be true, toxicity in the Illinois River has changed recently from a widespread, chronic problem to a more localized or episodic problem. Reduction of toxicity in surface sediments may reflect recent reductions in ammonia loading from sewage treatment plants in the Chicago area, although it is not clear whether the sources of ammonia in the porewaters are effluents, the deeper layers of sediments (as described above), or both.

We remind the reader that all the toxicity tests we conducted were short-term, acute tests. The fingernail clams, *Musculium transversum*, were exposed to sediment porewater for only 1 hour and then their filtering performance was tested in clean dilution water. The water flea, *Ceriodaphnia dubia*, was exposed to porewater for just 48 hours. The organisms in the waterways are exposed to contaminants for their entire life spans. In the past, more sensitive tests with fingernail clams have demonstrated toxicity even in downriver sediments, including fish and wildlife areas such as Peoria Lake and Quiver Lake (Sparks, Sandusky and Paparo 1981).

In addition to being a problem for the benthic invertebrates that fish feed upon, ammonia may be a problem for the fish themselves. In 1987, the U.S. Fish and Wildlife Service simulated resuspension of bottom sediments by boat- or wind-driven waves by stirring sediments in clean water, allowing the sediment to settle for 24 to 48 hours, then exposing larval fathead minnows, *Pimephales promelas*, to the water. Water mixed with surface sediments from the Chicago River and the Des Plaines River killed all the fish within 24 hours. Surface sediments from Lake Chautauqua, a bottomland lake and federal wildlife refuge along the Illinois River at Havana, killed 15% of the test fish in 96 hours; deeper sediments, taken at the 12- to 18-inch depth, killed 25%. Fish mortality correlated ($R = 0.71$, $P < 0.01$) with the concentration of un-ionized ammonia released from the sediment and both ammonia and fish mortality increased upstream toward Chicago. The Long-Term Research Monitoring Station (LTRM) at Havana started measuring ammonia concentrations in Anderson Lake, a floodplain lake of the Illinois River and a state fish and wildlife area, on 1 May 1990, 2 days after a fish kill. The total ammonia nitrogen concentration was 0.90 mg/l and the un-ionized ammonia nitrogen was calculated to be 0.36 mg/l at the temperature of 16.6$^{o}$ C and pH of 9.34. $NH_3$-N concentrations of 0.32 mg/l at 3-5$^{o}$ C and 1.35 mg/l at 24-25$^{o}$ C were acutely lethal to bluegill sunfish in bioassays conducted at our laboratory. The fish kill might have been caused by ammonia, if the un-ionized ammonia had peaked at higher concentrations before our samples were taken.

Elevated un-ionized ammonia concentrations might be triggered by resuspension of sediments or episodes of elevated pH resulting from

phytoplankton blooms. Plants remove carbon dioxide from the water, in the form of carbonic acid and bicarbonate, and thereby elevate the pH of the water, which in turn increases the proportion of ammonia existing the toxic, un-ionized form. The Havana LTRM station measured pHs as high as 10.12 in backwater lakes of the Illinois River in July 1990 and values between 9.0 and 10.0 occur fairly often. Episodes of acute ammonia toxicity thus may be occurring sporadically in places other than just the upper Illinois River, and it takes only one brief episode per year to kill or reduce populations that take many months or years to build up. Potential sources of ammonia or nitrogen, besides sewage plants and anaerobic sediments, include industrial plants (especially refineries and munitions plants), feedlots, and agricultural fields.

Although a general recovery does seem to be beginning in the Illinois River, with the return of fingernail clams in some areas where they have been absent at least 30 years and appearance of largemouth bass throughout the Illinois River proper, the pace and permanence of recovery still appears to be threatened by ammonia, even if the problem now turns out to be episodic instead of chronic. Reports of fingernail clam and mussel die-offs in the Upper Mississippi River and other rivers (Wilson et al. in press; Blodgett and Sparks 1987; Neves 1987) indicate that drastic population declines in macroinvertebrates that burrow in sediments are not unique to the Illinois River.

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Appendix Table A.1. Relationship between yeast concentration and % light transmittance measured by a spectrophotometer.

| Yeast mg/l | % Transmittance | | | |
|---|---|---|---|---|
| | x1 | x2 | x3 | $\bar{x}$ |
| 100 | 86.50 | 86.00 | 86.00 | 86.17 |
| 90 | 87.10 | 87.00 | 86.98 | 87.02 |
| 80 | 87.80 | 87.20 | 87.20 | 87.40 |
| 70 | 89.00 | 89.00 | 89.10 | 89.03 |
| 60 | 90.13 | 90.31 | 90.35 | 90.26 |
| 50 | 91.69 | 91.68 | 91.71 | 91.69 |
| 40 | 93.25 | 93.23 | 93.30 | 93.26 |
| 30 | 94.70 | 94.73 | 94.80 | 94.74 |
| 20 | 96.61 | 96.45 | 96.45 | 96.50 |
| 10 | 99.22 | 99.39 | 99.47 | 99.36 |

Yeast concentrations were calculated from a regression equation based on the above data:

Y = yeast concentration (mg/l)

T = % transmittance of light

$$Y = 676.378 - 6.788\ T$$